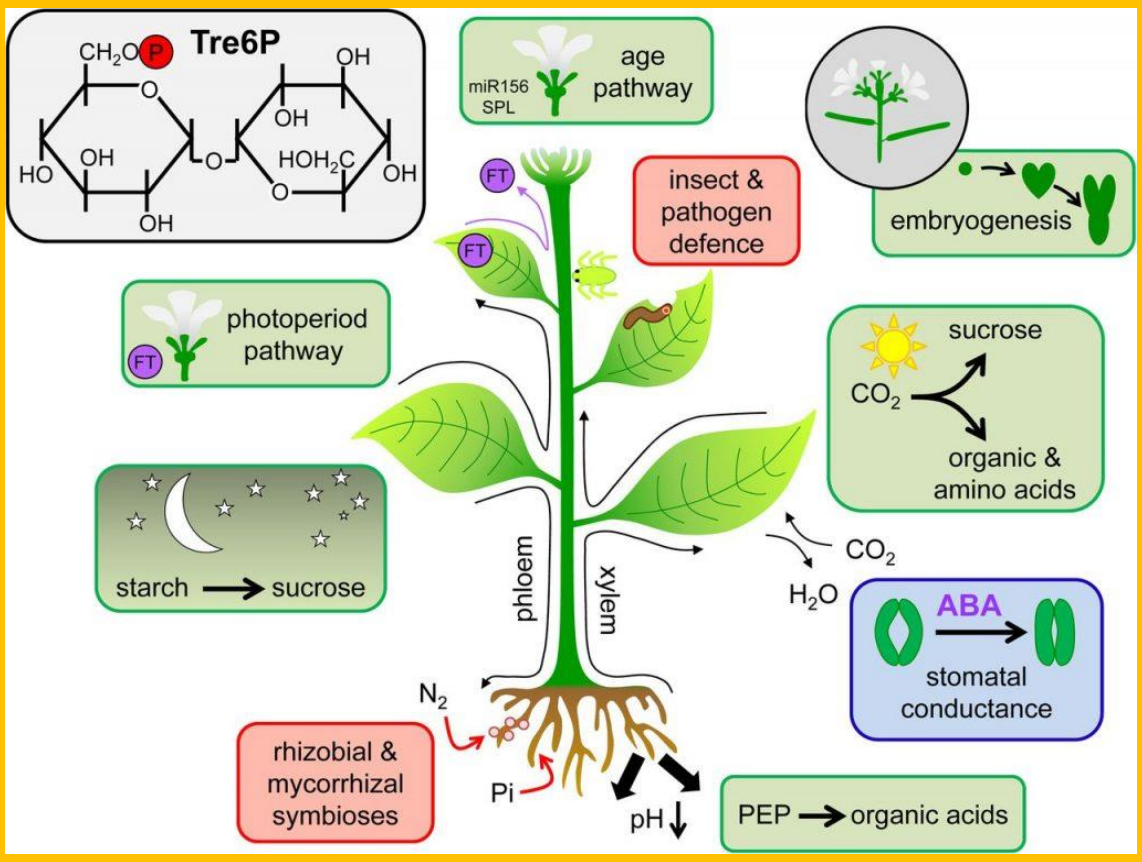
MSCBOT-601

M. Sc. III Semester

PLANT PHYSIOLOGY AND BIOCHEMISTRY



DEPARTMENT OF BOTANY
SCHOOL OF SCIENCES
UTTARAKHAND OPEN UNIVERSITY

MSCBOT 601

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BLOCK-1

PLANT SOIL WATER RELATIONSHIP

UNIT-1- PLANT SOIL WATER RELATIONSHIP

Contents:

1.1 OBJECTIVES

After reading this section you will know-

1. The physical and chemical properties of the water
2. The importance of water to the plants
3. What is soil plant atmosphere water movement
4. Why the water is a most important constituent for the plant life
5. How the plants absorb water from the soil

1.2 INTRODUCTION

Water is essential for life on the Earth. It is required by all the living cells for its different metabolic function and growth. Water on the Earth is present in two main forms, freshwater and marine water. Different organisms have their ability to survive in both fresh and marine water. But for terrestrial plants the water is a scarce resource. They absorb water through the roots but have to evaporate through the stomata in order to uptake the CO_2 for photosynthesis. They need to maintain the subtle balance between the absorption of water through the soil and its transpiration through the stomata. Let us discuss the properties of water that makes it essential for survival of life and how do the plant maintain the water balance between soil to the atmosphere.

1.3 THE STRUCTURE AND PROPERTIES OF WATER

We know that the water molecule consists of one oxygen and two hydrogen molecules. Oxygen is more electronegative than the hydrogen atom therefore it tends to attract the electrons of covalent bods between hydrogen and oxygen more towards the oxygen. It results in to the development of partial negative charge on oxygen molecule and partial positive charge on the hydrogen molecule. These charges make the water a polar molecule. Further, both the oxygen and hydrogen molecules can form the hydrogen bonds with other molecules or compounds having hydrogen, oxygen and nitrogen in their molecular structures. These properties provide the water molecules a prime status among the compounds present in the living beings.

Structurally, the water molecules are tetrahedral in shape. Hydrogen atoms with partial positive charge are present at the two corners of the tetrahedron and the other two corners have the lone pair of electrons which bear partial negative charge. These opposite charges on the water molecule make it ideal molecule for hydrogen bonding. Hydrogen bonds are weaker than the ionic and covalent bonds but it is responsible for many unusual properties of the water. Some important properties of water are as follows -

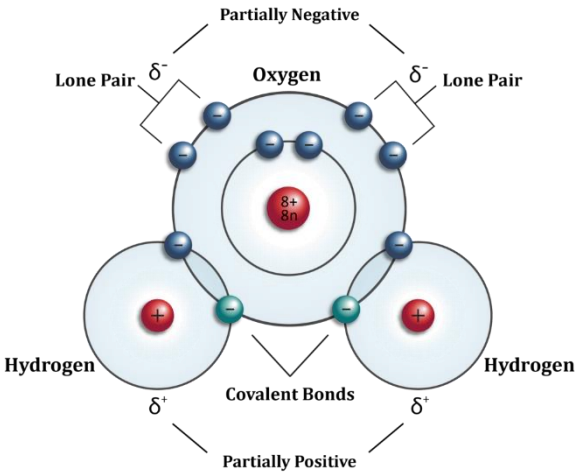


Figure 1.1: Structure of water molecule

1. Water is liquid at the room temperature in comparison to other hydrocarbons and ammonia despite having low molecular weight. Water molecule forms four hydrogen bonds with adjacent water molecules which results into very strong intermolecular interactions inhibiting the separation of the water molecules and escape as vapour (Fig. 1.1).
2. Water is an excellent solvent. It dissolves wide varieties of substances that too in greater quantities. This property of water is partially due to its small size, and highest known dielectric constant. The hydrogen bonding ability and polar structure makes water good solvent for the ionic compounds like sugar and proteins.
3. Water molecules have very high adhesive and cohesive properties in comparison to the other solvents. The attraction between the molecules of different compounds is called adhesion and in case of water it is due to the hydrogen bonding between the water and the other molecules. The attraction between the molecules of same compounds is called cohesion.
4. The cohesion provides water with the high tensile strength. It is defined as the maximum force present in per unit area that can withstand a continuous column of water before breaking. Studies have demonstrated that water can resist the pressure more negative than – 20 MPa (Mega Pascal) as opposed to the compression against its tensile strength.
5. The surface tension of water is higher than most of the other liquids that is also due to its high cohesive force between the water molecules. Together, adhesion and surface tension are responsible for causing the water pull up in to the capillary tube and provide the property of capillarity. Water in to the capillary move up the capillary tube until this upward force is balanced by the weight of the water column.
6. Water molecules have high specific heat capacity. This property is also due to the hydrogen bonding property of the water. The amount of heat required to raise the temperature of unit mass of a specific substance 1°C is called its specific heat. The high specific heat of the water is also due to the arrangement of the water molecules which allows the hydrogen and oxygen atoms to vibrate freely to absorb the large quantity of the heat energy.
7. Latent heat of vaporization of water is also high. It is the energy required to separate molecules from the liquid phase and convert them in to the gas phase. For water, the latent heat of vaporization at 25°C is 44 kJ mole $^{-1}$ which is highest for any known liquid. Most of the energy provided to the water is used to break the hydrogen bonds between the molecules. The latent heat does not change the temperature of the water molecule, but it decreases the temperature of the surface through which the water has evaporated.

8. Despite the hydrogen bonding in water molecules, the viscosity of the water molecules is very low. Viscosity is the property of resistance of molecules against the flow of a liquid. Viscosity decreases with increasing the temperature of the water.

1.4 SOIL PLANT ATMOSPHERE WATER MOVEMENT

Every living cell in the plants require the water. Plants absorb water through the roots from the soil and are transported across all plant body by different means. In soil and in the xylem, water moves by bulk flow in response to a pressure gradient i.e. hydrostatic pressure. When this water is transported across the membranes, the driving force is water potential difference across the membrane. Cellular processes depend on the transport of molecules both in to the cell and away from it. Diffusion and osmosis are the commonest method through which a cell transports the water in to the plants. During transpiration, the water moves out in vapour phase, primarily by the diffusion. By the process of transpiration, a continuous flow from the roots to the tip of the plant is established, thus contributing the formation of an integrated communication system of water among all plant parts.

1.4.1 Diffusion

Diffusion is the spontaneous movement of substances from regions of higher to lower concentrations. At cellular distances the diffusion is the most dominant mode of transport. Diffusion takes place by the random kinetic activities of molecules, ions or atoms. The net movement of molecules results into development of a pressure which is called Diffusion Pressure. The amount of pressure is inversely proportion to the average distance between the molecules or directly proportional to the concentration of the substances. It is also proportional to the temperature, i.e. increase in temperature increases the rate of diffusion.

In comparison to any solution of the solvent, the pure solvent shows maximum diffusion pressure (i.e. zero). When a solute is added in the solvent the chemical potential of the solvent decreases and a Diffusion Pressure Deficit (DPD) results. DPD of a solvent is proportional to the amount of solute added to it. All living cells sustain in aqueous environment and the phenomenon of diffusion is directly or indirectly involved in all physiological processes of a cell. Not only the water, but exchange of gases i.e. CO_2 and O_2 from the atmosphere and through the intercellular spaces also occurs by the diffusion. Diffusion is the main force behind absorption of water from soil by root hairs and for the loss of water through the transpiration.

1.4.2 Imbibition

Hydrophilic substances when placed in water, they absorb it and swell up. It is called imbibition. eg. Swelling up of seeds, resins, gums when placed in water, welling up of wooden doors during

rainy season and swelling up of rubber when place in kerosene oil. Imbibition involves both diffusion and capillarity. Substances which imbibe water are known as imbibants.

Imbibition results in increase in volume of imbibants but it is always less than the sum of the volume of dry imbibant and volume of water imbibed due to liberation of energy in form of heat. Greater the complexity of molecule more will be the imbibing capacity. eg. Proteins have greater imbibing capacity than fats and fats have more imbibing capacity than carbohydrates. Imbibition is the first process that occurs when root hairs absorb water from the soil. The hydrostatic pressure that develops in an imbibant when it is immerged in water is called imbibitional pressure. It is a powerful force. Dry wooden logs placed in rock crevices during summer season can break the rocks in rainy season after the imbibition.

Two conditions are necessary for imbibition –

1. A diffusion pressure gradient must exist between the imbibant and the substance imbibed.
2. A certain affinity must exist between components of the imbibant and the imbibed substance.

Imbibition pressure is analogous to osmotic pressure in that it represents the potential maximum pressure that an imbibant will develop if submerged in pure water. It can be expressed as –

$$DPD = IP - TP$$

(DPD = Diffusion Pressure Deficit, IP = Imbibition Pressure, TP = Turgor pressure)

No turgor pressure develops in an unconfined imbibant and above expression under these conditions simplifies to –

$$DPD = IP$$

The rate of imbibition is affected by temperature and osmotic pressure of the substances to be imbibed. An increase in temperature increases the rate of imbibition but increase in osmotic pressure lowers the imbibitional pressure.

1.4.3 Osmosis

Osmosis is the diffusion of water across the selectively permeable membrane. In plant cells the membrane is selectively permeable i.e. it permits the movement of water and other small uncharged molecules move across it than the other large and highly charged molecules. If in the cell, the concentration of the solutes is higher than the solution of the surrounding medium, the water will diffuse inside the cell, but solute cannot cross out of the membrane. This net movement of water across the selectively permeable membrane is called **Osmosis**.

The Osmotic pressure (π) of a solution is defined as the positive pressure exerted on the solution necessary to prevent a net flow of water between solution and its pure solvent, when these are separated by a perfect semi-permeable membrane.

$$\pi = CRT,$$

Where, π is the osmotic pressure, C the molar concentration of solution, R is gas constant (0.0357 litre calory per degree centigrade) T is the absolute temperature. It is usually expressed

in litre atmospheres. But practically, no isolated solution can possess an osmotic pressure since it is only demonstrated in presence of pure solvent. The Solute potential or **Osmotic potential** (Ψs) of a solution, is equal in magnitude but opposite sign to the π. Ψs (Ψ = psi) is an important factor for plant cell in determining its water relationship with other cells and soil. Osmosis is measured by the device **Osmometer**.

1.4.3.1 Osmotic relations of plant cell

When a solution has got the same osmotic pressure as that of the cell sap, it is called an **Isotonic** solution. On the other hand, the **Hypotonic** solution has relatively low concentration than that of the cell sap and water enters the cell form the surrounding solution. A **Hypertonic** solution has a higher concentration of solute particles than the cell sap and water ooze out of the cell resulting in to the decrease in turgor pressure. The hydrostatic pressure exerted by the cell sap or cytoplasm and ultimately on the cell wall is known as **Turgor Pressure (P)**. The turgor pressure develops due to **endosmosis** and is responsible for pressing the plasma membrane against the cell wall. The turgor pressure is also known as pressure potential (Ψp) and it is included as component of water potential. The counter equal and inversely directed pressure exerted by the cell wall on the cell cytoplasm is known as **Wall pressure**. The turgor pressure is always equal to the wall pressure. In a flaccid cell, the turgor pressure is zero therefore, wall pressure is also zero. In a fully turgid cell, the turgor pressure is maximum and wall pressure is also maximum.

In hypertonic solution, the plant cell loses turgor and if it is examined under the microscope, the characteristic shrinkage of the protoplasm is observed. This shrinkage of the protoplasm, due to hypertonic solution, is known as **Plasmolysis** and the cell is known as plasmolyzed cell. The stage at which the protoplasm just starts to shrink and starts coming apart from the cell wall is called **Incipient Plasmolysis** state. When the entire water diffuses out by **exosmosis**, vacuole disappears, protoplast comes to lie as a spherical ball and the space between the cell wall and protoplasm is filled with external hypertonic solution. This stage is called as Evident plasmolysis.

If a plasmolysed cell is transferred to pure water or hypotonic solution, water will diffuse into the cell and the protoplasm gradually becomes turgid and recovers its normal shape. This phenomenon is known as **Deplasmolysis**. The deplasmolysis shows that cell is alive, however, prolonged plasmolysis is irreversible and results in death. Plasmolysis indicates that cell wall is a permeable layer and plasma membrane is selectively permeable membrane.

1.4.4 Diffusion pressure deficit

Diffusion pressure deficit or DPD is the force with which water enters in to a plant cell. It can also be defined as the difference of diffusion pressure of pure water and water in a solution. DPD is a parameter which indicates water absorbing capacity of the cell. It is the difference of Osmotic pressure and turgor pressure.

Ψd = Ψp – Ψt

Where, Ψd = DPD, Ψp = Osmotic pressure, Ψt = Turgor pressure

In a flaccid cell Ψt = 0 therefore, Ψd = Ψp.

In a fully turgid cell, Ψp = Ψt, hence, Ψd = 0

Therefore, DPD is maximum in a flaccid cell and minimum in fully turgid cell. The actual movement of water occurs along the DPD gradient, i.e. from the region of lower DPD to the higher DPD region. DPD can also be defined as the difference of free energy status of pure water and water in a solution at the same temperature and pressure.

1.4.5 Water potential

The free energy status of the water or the chemical potential of water is known as **Water Potential**. It is a relative property and indicates the difference in the free energy status of given sample of water as compared to the free energy status of pure water in reference stage. The unit of water potential is joule mol^{-1} and it is symbolized as Ψw. Water potential is highest for pure water i.e. zero. The water potential concept was formulated by Otto Renner in 1915.

In plant cell, the water potential depends on the solute concentration, pressure and gravity. Water potential of solutions may be divided in to its components as below-

$$\Psi w = \Psi s + \Psi p + \Psi g$$

Ψs indicates the **solute potential**. It is the effect of dissolved solutes in the water potential. Solutes reduce the free energy of water i.e. it dilutes the water andits value is negative. Mixing of solutes in the water increases the entropy or disorder of the system and hence lowers the free energy. Thus, osmotic potential is independent of the specific nature of the solute. For dilute solutions of non-dissociating substances like sucrose the osmotic potential can be calculated as –

$$\Psi s = -RTcs$$

Where, R is gas constant (8.32 J mol^{-1} K^{-1}) T is absolute temperature in K° and c_s solute concentration of the solution in mol L^{-1}. The minus sign indicates that dissolved solutes reduce the water potential of the solution.

Ψp is the hydrostatic pressure or pressure potential of the solution. Positive pressure increases the water potential and vice-versa. In plants, the positive refers to as **turgor pressure**. In xylem, and in between plant cells, a tension or negative hydrostatic pressure can develop where the value of Ψp can be negative.

$$\Psi w = \Psi p + \Psi s$$

$$Or, \Psi w = P - \pi$$

$$as\ \pi = -\Psi s$$

(P = Turgor pressure, π = Osmotic pressure)

Hydrostatic pressure is measured as the deviation from the atmospheric pressure. Water in the reference state is at atmospheric pressure, so at the standard state Ψp = 0 MPa (Mega Pascal).

Therefore, the value of Ψp for pure water in open vessel is 0 MPa, even though its absolute pressure is about 0.1 MPa (1 atmosphere).

Ψg is the gravitational potential. It depends on the height of the water above the reference state water. Gravity causes water to move downwards unless the force of gravity is opposed by an equal and opposite force. A vertical distance of 10m causes about 0.1MPa change in water potential.

Besides above, water in dry soil and in plant tissues with very low water content like seeds, woods and water adsorbed on hydrophilic substances, matric potential **Ψm** is also taken in to the consideration. In these substances the water exists as thin layer of film bound to the surface of substance by electrostatic attraction. Bound water is that fraction of water that gets adsorbed on colloidal surface by hydrogen bonding. This fraction of water doesn't function as free water as such as it doesn't get frozen or evaporated. The matric potential is important in cases like developing or germinating seeds and succulent plants. Many workers, in general, exclude the matric potential

Water potential is numerically equal to the DPD but its value is negative. In an experimental plant cell we can ignore the values of Ψg and Ψm, therefore -

$$\Psi w = \Psi p + \Psi s$$

Or, $\Psi w = P - \pi$, as $\pi = - \Psi s$ (here, P = Turgor pressure, π = Osmotic pressure)

When such a cell is immersed into the pure water, then water will move into the cell until Ψp equals to the Ψs and total water potential will be zero. This is the fully turgid condition when water absorption stops, where

$$\Psi w = 0 = \Psi p + \Psi s$$

Or, $- \Psi s = \Psi p$

Or, $\pi = P$

When the same cell is placed into the solution of low solute potential, water will come out of the cell. At the moment, when the membrane stops to exert pressure against the cell wall, Ψp becomes zeroand Ψs becomes equal to Ψs. This is the point of incipient plasmolysis.

When, $\Psi p = 0$,

$\Psi w = \Psi s \Psi w$

Or, $\Psi w = \pi$

Therefore, movement of water occurs in response to Ψw of cell, not just to Ψw. The water potential can be negative, zero or positive.

In plants, the water potential is almost always negative. In soil-plant-air system, under most of the conditions, when relative humidity is less than hundred percentage, the water potential is highest in the soil and lowest in the atmosphere, with intermediate values in various parts of the plant. There establishes a gradient from the soil, through the plant, to the atmosphere but with varying components. In the soil, P = 0, π is slightly negative, because soil solution is dilute. In xylem, water contains few solutes so π is only little slightly negative, but here, water may be under tension (due to negative P) hence, water potential is more negative in xylem than the soil water and the water moves up into the plants from the soil. In leaves, where the cells contain more solutes in the cytoplasm, the π is more negative, water moves into them and makes a positive pressure (P) but water potential in the cell remains more negative than in the xylem. Atmospheric water potential is even more negative, so water tends to move out of the leaves in to atmosphere through the stomata.

During rainy season or heavy dew, when relative humidity in near the hundred percentage, a few species may actually absorb the water from atmosphere through the leaves and build up a positive pressure (P) and hence build the positive pressure in xylem also. But this condition is rare. Therefore, in plants the water potential is usually negative.

1.4.5.1 Determination of water potential

Water potential is the most reliable property that can be measured in soil-plant-air system. We know that at equilibrium, the water potential in plant cell is zero. Thus, a plant part can be introduced into a closed system and when the equilibrium is achieved the water potential can be determined for any part of the plant system.

In **Tissue-Volume method**, a sample of tissue is placed in each of the series of solution of varying but known concentration of solutes like sorbitol, glycol, mannitol or polyethylene glycol. The object is to find the solution in which the tissue volume does not change, indicating no gain or loss of water, which indicates that the tissue and solution were in equilibrium since the beginning. Hence, the water potential of the tissue must be equal to that of the solution.

In other method, the water potential of the tissue sample can be measured by measuring the changes in weight of tissue rather than changes in volume at equilibrium. This method is known as Gravimetric method.

In another approach, instead of measuring the changes in tissue, one can measure the concentration of test solution. If it becomes less concentrated, the tissue will have lost the water. It is a better approach than measuring the tissue volumes.

In **Chardakov's method**, the changes in concentration of external test solution are measured using dye like methylene blue, instead of changes in tissue. In this method, two graded series of solutions of proper osmoticum like sucrose, mannitol or PEG are taken in duplicate set. For

control series, the second tube of each set is coloured slightly by dissolving methylene blue which does not change osmotic potential significantly. Tissue samples are placed in test tubes that contain solutions of equivalent concentration but no dye. Time is allowed to exchange of certain amount of water, about 5 to 15 minutes. Then tissue is removed and a small drop of equivalent coloured solution is added into the test tube. If the coloured drop rises, the solution in which the tissue was incubated has become more dense, indicating that the tissue has taken up the water, in this case, tissue had a lower water potential than the original solution. If the drop sinks, the solution has become less dense, having absorbed water from the tissue, then the solution has lower water potential than the tissue. If the drop diffuses evenly into the solution without rising or sinking, then no change in concentration has occurred and the water potential of the solution is equal to that of the tissue. This point is called null point. At this stage turgor pressure P = 0, therefore, $\Psi w = \pi$.

Another method to determine the water potential is **vapour pressure method**. It is done by using psychrometer or similar device. In this method the vapour pressure of the air in equilibrium with a tissue in an enclosed chamber may be determined directly.

Water potential of a leafy twig or shoot can be measured by the **pressure bomb technique**. The pressure required to force out the xylem sap from the cut end is equivalent to the water potential existing in the tissue before the cutting. This value is equal in magnitude but opposite in sign to the tension in the intact xylem.

Water potential can also be determined by measuring the **relative water content** (RWC) of a particular tissue. RWC is the water content of a tissue compared with the water content of the same tissue in fully turgid condition.

$$\text{RWC} = \frac{Wf - Wd}{Wt - Wd} \times 100$$

Where, Wf= initial fresh weight of the tissue, Wt = turgid weight (final), and Wd = dry weight of the tissue.

1.4.6 Aquaporins

Porins are a class of membrane proteins that are found in cell membranes of all living organisms. These are non-selective channels which are characterized by the β-pleated sheet of protein structure. These protein units in plants, the aquaporins are membrane protein channels which control the selective movement of the water (Fig. 1.2). The structure of aquaporins is highly conserved among plants, microbes and animals. Four sub-units (tetramers) of polypeptides associate to form a single plant aquaporin. These protein subunits fold within the membrane in such that the hydrophobic amino acids are on the outer side of the pore and interact with the hydrophobic fatty acids of the lipid bilayer whereas the hydrophilic amino acids are in the inner side of the pore and interact with water molecules as they move through the pore form one side of the membrane to the other. Aquaporins can be open or closed to regulate the

movement of water across the membrane. This control is achieved through the cytoplasmic pH, the concentration of divalent cations like Ca^{+2} and by aquaporin protein phosphorylation. There are two possible pathways for the movement of water across a membrane – one through the lipid bilayer itself and the other pathway through an aquaporin. The rate of water movement through a lipid bilayer is faster through the aquaporin than a membrane that contains lipid only. Since, the aquaporins are gated channels, they provide better control for the movement of water intra-cellularly and inter-cellularly as well. Thus, aquaporins are important in regulating the osmotic properties of plant cells and help in osmoregulation.

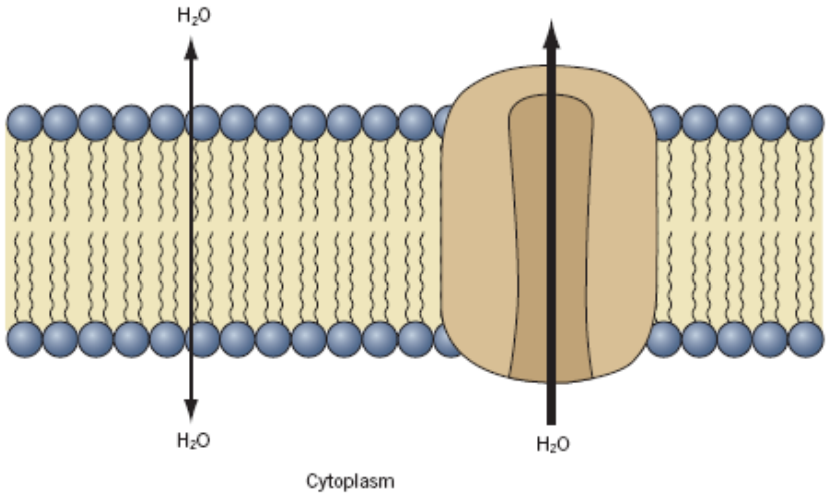


Figure 1.2: Transport across the membrane through lipid bilayer and through the aquaporin

1.4.7 Transpiration

Loss of water through the aerial parts of the plants is known as Transpiration. Transpiration is a vital process and evaporation is a physical process. Transpiration is regulated by stomata which are tiny prose surrounded by a pair of reniform guard cells. In members of Poaceae the guard cells are dumble shaped.

Transpiration takes place through the following parts of the plants-

1. Cuticular transpiration – A small amount of water (0.5-5%) is lost as vapours from the general surface of herbaceous stem and leaf through the cuticle. It is called as cuticular transpiration.

2. Lenticular transpiration – Very small amount of water (up to 0.5%) is lost through lenticels of the woody stem. In deciduous woody tree in autumn, only lenticular transpiration occurs.

3. Stomatal transpiration – Maximum amount of water is lost (up to 99%) as vapours through the stomata.

Stomata are surrounded by guard cells which are only epidermal cells in terrestrial plants which contain chloroplast. Inner wall of the guard cells is thicker than the outer wall. Sometimes the epidermal cells surrounding the guard cells are different from the other epidermal cells and are called as Subsidiary cells.

Usually guard cells regulate the opening and closing of the stomata. In most of the plants, the stomata open during the day, but in succulents like *Bryophyllum*, *Opuntia*, Pineapple the stomata open during the night.

1.4.7.1. Theories of opening and closing of stomata

Stomata open and close due to change in volume and shape of the guard cells which occurs due to change in turgor (Fig. 1.3). Change in turgor is brough about by the change in osmotic pressure. When osmotic pressure increases DPD of the guard cells increases. They absorb the water and their turgor pressure increases. Now outer thin wall expands but inner thinner wall becomes concave and are pulled apart resulting in the opening of the stomata.

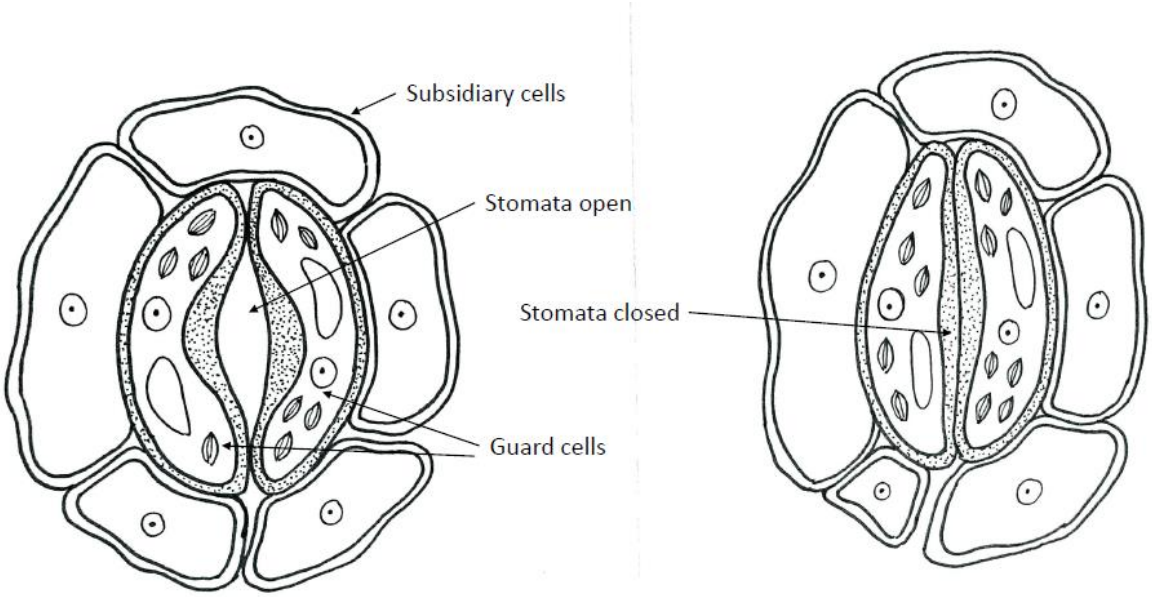


Figure1.3. Structure of stomata

Von Mohl suggested that during the day chloroplast of the guard cells perform photosynthesis, sugar is formed, osmotic pressure increases and stomata are open. In the night, the photosynthesis does not occur hence stomata are closed.

Lloyd suggested that during the day starch is hydrolysed in to sugar, which increases the osmotic pressure and stomata open. In the dark, sugar condenses to form starch and this decreases the osmoticum and stomata are closed.

Sayre suggested that stomata open in neutral or alkaline medium and close in acidic medium

Scarth suggested that CO_2 given out by respriration during the day is used in photosynthesis, therefore, pH remains high. In the night photosynthesis does not occur and CO_2 accumulates resulting in low pH. At high pH starch changes into sugar therefore, stomata open during the day. At low pH sugar changes into starch therefore, stomata close in night.

Hanes isolated enzyme Phosphorylase from the chloroplast of the guard cells.

Yin and Tung suggested that during day enzyme Phosphorylase changes starch and inorganic phosphate in glucose 1 phosphate and stomata open. In the dark, reversible reaction occurs and stomata close.

Steward suggested that formation of glucose 1 phosphate does not result in appreciable change in osmotic pressure. Osmotic pressure changes when glucose 1 phosphate changes into Glucose and Phosphate. In the night, glucose is converted again in to starch and inorganic phosphate so the stomata are closed.

Zelitch suggested that in low concentration of CO_2 during the day glycolate is formed in guard cells. It forms sugar which increases the osmotic pressure and it also supplies ATP required for the opening of stomata. Therefore, stomata open during the day. In night, glycolate formation does not occur and stomata are closed.

Levitt (Active K^+ transport mechanism) – suggested that stomata open due to accumulation of K^+ in the guard cells. He suggested that during the day organic acid namely Malic acid is formed. Malic acid dissociates into H^+ and $RCOO^-$ ion. H^+ are exchanged with K^+ ion present in the subsidiary cells because, K^+ ion is more electropositive than the H^+ and it pairs more strongly with malate ions. When K^+ accumulates, stomata open. In the night breakdown of the malate occurs, K^+ diffuses out and stomata are closed.

Nishida suggested that CO_2 released in respiration in night combines with water to form organic acid. Organic acid increases osmotic pressure and stomata open. During the day, break down of the organic acid takes place and released CO_2 is used in photosynthesis, therefore stomata close.

Pallas and Ehrler suggested that stomata in succulents are initially closed in the evening. Anaerobic respiration now begins, phosphoenol pyruvate formed in glycolysis combines with the CO_2 to form malic acid. Malic acid dissociates into H^+ and $R(COO)_2^-$. H^+ are exchanged with K^+ ion and stomata open. During the day decarboxylation of malate ions takes place and stomata are closed.

1.4.7.2 Importance of transpiration

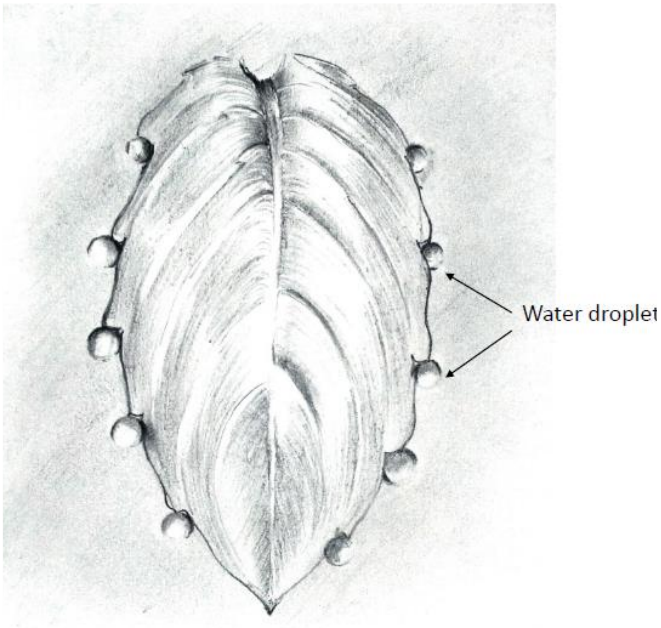
1. Transpiration is a means to achieve thermoregulation, because a transpiring plant has temperature 6-7 °C lower than the temperature of a wilting leaf. Overheating could result in chloroplast damaging and to stop photosynthesis

2. Due to transpiration, continuous water absorption and transport takes place together with the ions of mineral salts dissolved in it.

3. Transpiration results in continuous flow from the roots to the tip of the plant, thus contribution to the formation of an integrated communication system between all plant organs.

Antitranspirants- Certain substances like colourless plastics, low viscosity waxes, silicon oil, phenyl mercuric acetate, Bordeaux mixture, abscisic acid and CO inhibit transpiration by causing stomata closure. These are called as antitranspirants.

1.4.8 Guttation



Coming out of water in the form of drops through the uninjured tips at the margin of leaves is known as guttation (fig 1.4). Guttation can be seen in the leaves of tomato, *Cyanodon*, *Oxalis*. Guttation is observed only in plants having rate of water absorption higher than the rate of transpiration i.e. guttation occurs when atmosphere is moist and humid. Therefore, guttation occurs due to root pressure. Guttation occurs through the specialized pores present on the margin and tips of the leaves called as hydathodes or water stomata. Guttation water is not pure, it contains organic and inorganic solutes. So, guttation can be called as an excretory process to eliminate excess of water.

Fig.1.4. Guttation through leaf margin

1.4.9 Soil water

Water is important component of every type of soil. In the soil, water comes through the percolation of rainwater, melting of the snow and by upward movement of ground water by capillary action. Depending on the physical forces and the mode of retention, soil water is categorized as follows –

1. Hygroscopic water is the water which is which is held on the surface of the colloidal particles like clay, by the dipole orientation of its molecules. It forms a very thin film (3-10 layers of molecules of water) around the soil particles. These water molecules are held with a great force due to which it is not available for the plant system.
2. Capillary water is the water which is present as a thin film between the soil particles. It is held due to the capillary action of the inter-particular spaces. The amount of capillary water in the soil varies with the surface tension of the water film, soil structure, texture and organic matter. The capillary water can evaporate as the water molecules are free and mobile. This water is available to the plant system.
3. Gravitational water is the water which percolates to deeper layers into the soil surface under the influence of gravity and is not available to the root system of the plants.
4. Combined or bound water is the water which is present in to the soil in small amount in the form of chemically bound to the soil elements. This type of water cannot be detached form the soil elements except strong heating. This water is also not available to the plants.

There are two important factors of soil water regarding its availability to the plants, one is the amount of water stored in the soil and second is its relationship with soil water potential. The water storage largely depends on the pore size. The pore size larger than 30 µm like sandy soil hold little water as water easily drains out while pore size smaller than 0.2 µm hold water so firmly that water hardly drains out. Therefore, for plants the absorption of water is easy when the soil pore size ranges between 0.2 to 30 µm in diameter.

The capacity of soil to hold the maximum amount of water against the pull of gravity is called its Water holding capacity or **Field capacity**. It comprises the capillary water, hygroscopic water, combined water and the water vapour. It is the water content of the saturated soil. It is constant for a given soil and differs from one soil type to the another based on soil texture, structure, and organic content. Field capacity ranges between 0.01 – 0.03 MPa. As plants absorb water continuously from the soil the amount of capillary water is reduced gradually, and a point is reached when the plants cannot withdraw the water. As a result, the plants begin to wilt due to loss of turgor as transpiration continues, though the soil particles still retain certain amount of water. This stage is termed as **wilting point**. If the plants still absorb the water, the capillary water is further reduced reaching a point when plants do not regain their turgidity unless water is applied to the soil. This is termed **permanent wilting**. At this stage percentage amount of water held by the soil is termed **Permanent Wilting Percentage** (PWP) or **Wilting Coefficient** (WC). At PWP, soil water potential is equal to or less than minimum osmotic potential of plants so that they cannot take up water from the soil.

For mesophytes, PWP is about -1.5 MPa but it may range between -1 to -8 MPa. The amount of water between FC and PWP is called available water which can be absorbed by the plants through roots. In soil, hydrostatic pressure is generally negligible,

and matric potential is the most important component of the soil water potential. But for saline soils, the osmotic potential is also an important component that matters for absorption of water by the plants.

	Soil		
	Sand	**Loam**	**Clay**
Pore space (total %)			
$> 30\ \mu m$	75	18	6
0.2- 30 µm	22	48	40
> 0.2µm	3	34	53
Water content (% of volume)			
Field capacity	10	20	40
Permanent Wilting Point	5	10	20

Table 1. Pore size distribution and soil water content in three types of soils based on texture (from Lambers et al. 1998)

1.4.10 Importance of water to the plants

Water plays crucial role in plant life. It is the most important compound of the protoplasm which is essential for all the vital processes and to maintain the physical state of the plant cell. Plants start their life through the germination of seeds for which water is an essential requirement. It is a basic solvent for mineral salts and organic compounds as well as the dispersal medium for the different metabolites. Hydrophilic and hydrophobic interactions of different biological molecules like lipids, protein and carbohydrates with water is essential for the structural integrity and functions of the cell and its organelles.It is an important component for the photosynthesis where during photolysis of water evolution of oxygen takes place. But for gaseous exchange plants require the stomata to be open which results into loss of water. Due to sedentary life, the plants regulate their temperature through the transpiration which requires the water to be absorbed continuously. Plants are required to maintain the internal hydrostatic pressure called Turgor pressure, which is essential for growth and many physiological processes like stomatal opening, transport through the phloem. Water is also essential for absorption of minerals through the roots of the plants. In nonlignified herbaceous plants the turgor pressure is required for mechanical stability and rigidity in absence of non-lignified tissues.

1.5 SUMMARY

In this unit we have discussed the physical and chemical properties of the water, the importance of water to the plants, forces involved in soil plant atmosphere water movement, concept of diffusion, osmosis, and water potential and properties of soil water.

Let us sum up key points of this chapter –

1. The unique property of water is due to its ability to form the hydrogen bonds. This bond forming ability of water is derived through its polar nature and tetrahedral shape.
2. Water is an excellent solvent and has very high specific heat, latent heat and tensile strength.
3. Plants absorb water mainly through their roots and are carried through the plant and are lost by transpiration from the leaves.
4. Diffusion is the spontaneous movement of molecules or ions from regions of higher to lower concentrations. At cellular distances the diffusion is the most dominant mode of transport.
5. Osmosis the movement of water molecules from higher to lower concentration of water across the semipermeable membrane.
6. DPD is difference of diffusion pressure of pure water and water in a solution
7. Free energy status of a given sample of water is called water potential. Water potential depends on solute potential, pressure potential and gravitation potential.
8. Plant cell typically have negative water potential and movement of water in cell occurs along the water potential gradient.
9. Water potential can be determined by Tissue-volume method, Chardakov method, vapour pressure method, pressure bomb method, and by relative water content method.
10. The capacity of soil to hold the maximum amount of water against the pull of gravity is called its Water holding capacity or Field capacity. It comprises the capillary water, hygroscopic water, combined water and the water vapour.
11. Plants can absorb only capillary water.
12. At Permanent Wilting Point, soil water potential is equal to or less than minimum osmotic potential of plants so that they cannot take up water from the soil.
13. Besides normal growth and development, plant require huge amount of water for transpiration and maintaining their body temperature.

1.6 GLOSSARY

Apoplast: The non-cytoplasmic continuity in the plant body, which includes cell wall space, xylem vessels and tracheids etc.

Aquaporins: Protein channels that control the selective movement of water across the membranes.

Field capacity: The water that remains in capillary pores of soil after gravitational water goes down the soil.

Free energy: Energy that is available to do work.

Hypertonic solution: A solution with a higher solute content than a cell or another solution and hence, more negative osmotic potential.

Hypotonic solution: A solution with a lower solute content than a cell or another solution and hence, less negative osmotic potential.

Matric potential: The contribution to water potential by the adsorption of water to solid surfaces.

Osmoregulation: The process of regulating the osmotic properties of plant cell.

Osmosis: The property of water passing through a semipermeable membrane with the tendency of equalizing the water potential in the two different sides of membrane.

Osmotic potential: The change in free energy or chemical potential of water produced by the solutes; it is also known as solute potential.

Permanent wilting point: The soil water content below which a plant is unable to extract the sufficient water to maintain turgor.

Plasmolysis: A condition when the protoplast sinks away from the cell wall due to loss of turgor.

Soil-plant-atmosphere continuum: The integrated flow of water from the soil through the plants and into the atmosphere.

Stomata:Tiny pores on outer plant surface surrounded by dumbbell shaped cells called guard cells which regulate the movement of water through the plant surface

Symplast: Protoplast continuity through the plasmadesmata.

Turgor pressure: The pressure that arises from the force exerted outwardly against the cell walls by the expanding protoplast.

Wall pressure: The inward pressure exerted by the cell wall against an expanding protoplast which is equal but opposite to turgor pressure.

Water potential: Free energy status of water in solution which is algebraic sum of pressure potential, solute potential and gravitational potential.

1.7 SELF ASSESSMENT QUESTIONS

1.7.1 Very short answer questions

1. In which two forms the water is presenton the Earth?
2. The shape of water molecule is?
3. Most of unusual properties of water is due to which bonding?

4. At cellular distances the most dominant form of water transport is?
5. Diffusion of water across selectively permeable membrane is called?
6. Measurement of osmosis is done by?
7. A solution having osmotic pressure similar to that of cell sap is called?
8. Free energy status of water in a solution is called?
9. Protein channels regulating movement of water across the membrane are called?
10. In soil, the water which is available to the plants is called?
11. When osmotic pressure increases the rate of imbibition is?
12. Active K^+ transport mechanism for transpiration was given by?

Answer: 1. Freshwater and marine water, 2. Tetrahedral, 3. H-bonding, 4. Diffusion, 5. Osmosis, 6. Osmometer, 7. Isotonic, 8, Water potential, 9. Aquaporins, 10. Capillary water, 11. Decreased, 12. Lewitt

1.7.2 Multiple choice questions

1. Living organisms can survive in:
   a) Freshwater b) Marine water
   c) Both a and b d) None of the above

2. In water molecules, the hydrogen bond can be formed by
   a) Hydrogen atom b)Oxygen atom
   c) Both Hydrogen and Oxygen atoms d) None of the above

3. How many hydrogen bonds can be formed by a water molecule
   a) One b) Two
   c) Three d) Four

4. Attraction between the molecules of same compound is called
   a) Adhesive force b) Cohesive force
   c) Ionic force d) Covalent bond

5. The force responsible to withstand a continuous column of water before breaking is called
   a) Tensile strength b) Pliable strength
   b) Shear strength

6. The amount of heat required to raise the temperature of unit mass of a specific substance is called
   a) Latent heat b) Specific heat
   c) Heat of vapourization d) None of the above

7. Which one is the best correct among followings about the water

a) It has high latent heat
b) It has high specific heat
c) It has high heat of vapourization
d) All of the above

8. The bulk flow of water in soil and xylem is due to
   a) Diffusion
   b) Osmosis
   c) Hydrostatic pressure
   d) Osmotic pressure

9. Spontaneous movement of substances from regions of higher to lower concentrations is called as
   a) Imbibation
   b) Osmosis
   c) Wall pressure
   d) Diffusion

10. Osmosis is the diffusion of water across the membrane, which should be
    a) Permeable membrane
    b) Non-permeable membrane
    c) Selectively permeable membrane
    d) Any of the above

11. The hydrostatic pressure exerted by the cell sap and ultimately on the cell wall is known as
    a) Turgor pressure
    b) Wall pressure
    c) Imbibational pressure
    d) Atmospheric pressure

12. Which among the followings indicates water absorbing capacity of the cell
    a) Hydrostatic pressure
    b) DPD
    c) Osmotic potential
    d) Imbibational pressure

13. The water potential concept was formulated by
    a) Otto Renner
    b) Abe Nollet
    c) Johanson
    d) Ingenhausz

14. Water potential is highest for
    a) Pure water
    b) Water present in cell sap
    c) Water present in phloem
    d) Water present in xylem

15. How many polypeptides are involved in formation of one aquaporin channel –
    a) One
    b) Two
    b) Three
    d) Four

16. The shrinkage of the protoplasm, due to hypertonic solution, is known as –
    a) Exosmosis
    b) Endosmosis
    c) Plasmolysis
    d) Ambolism

17. In plants, the value of water potential is usually –
    a) Negative b) Positive
    c) One d) More than 1

18. Water potential can be measured by
    a) Tissue volume method b) Vapour pressure method
    c) Pressure bomb technique d) All of the above

19. Which among the following water is absorbed by the plants
    a) Hygroscopic water b) Gravitational water
    c) Combined water d) None of the above

20. Which among the followings is not the component of field capacity
    a) Hygroscopic water b) Gravitational water
    c) Combined water d) Capillary water

21. Imbibition pressure is affected by
    a) Temperature b) Osmotic pressure
    c) Both of the above d) None of the above

22. Coming out of water in the form of drops through the uninjured tips at the margin of leaves is known as
    a) Transpiration b) Guttation
    c) Imbibation d) None of the above

Answers:1.c, 2 c, 3.d, 4.a, 5.a, 6.b, 7.d, 8.c, 9.d, 10.c, 11.a, 12.b, 13.b, 14.a, 15.d, 16.c, 17.a, 18.d, 19.d, 20.b. 21. c, 22. B

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1.10 TERMINAL QUESTIONS

1.10.1 Long answer type questions

1. Describe the different physical and chemical properties of the water?
2. Give a detailed account on osmotic relations of plant cell.
3. Describing different components of water potential explain its importance with the reference to the plants.
4. What is water potential? How it is measured?
5. Describe in detail about the different forms of water in thesoil water?
6. What do you mean by transpiration? Why it is called as necessary evil to the plants?

1.10.2 Short answer type questions

1. Write short note on soil plant atmosphere water movement.
2. What is diffusion? What is its importance to the plants?
3. Write comments upon the osmosis.
4. What is diffusion pressure deficit?
5. What are aquaporins? How do they help in water movements in plants?
6. Write a short note on importance of water to the plants.
7. What do you mean by imbibition? What is its significance to the plants?
8. Why the transpiration is important for the plants?

UNIT-2- MINERAL NUTRITION

Contents:

2.1 OBJECTIVES

After reading this unit students will be able:

- to acquire knowledge about the mineral nutrition
- to understand about the macronutrients and micronutrients
- to know about the techniques to Study the Role of Mineral Nutrients

2.2 INTRODUCTION

Plants use inorganic minerals for nutrition and its roots absorb minerals in the form of ions from the soil, while the minerals naturally remain in the soil as salts. Mineral nutrition in plants is the phenomenon in which the plant roots absorb various minerals or nutrients that are essential for cell growth, metabolism and reproduction. The process of absorption, translocation and assimilation of nutrients by the plant is known as mineral nutrition.

De Saussure (1804) recognized that mineral elements present in the soil are important for the growth and development of plant. Liebig (1840) for the first time gave evidence about the functions of these plant elements.

Plants need water to support cell structure, for metabolic functions, to carry nutrients, and for photosynthesis. Plant cells need essential substances, collectively called nutrients, to sustain life. There are seventeen essential nutrients for plant growth. These nutrients are required in different quantities and concentrations. Nutrients that plants require in large amounts are called macronutrients. Macronutrients include oxygen, carbon, nitrogen, hydrogen, phosphorous, calcium, potassium, magnesium and sulphur. Those elements required in smaller quantities are known as micronutrients. Micronutrients are iron, copper, manganese, zinc, molybdenum, boron and chlorine.

Plants require both organic and inorganic compounds for proper growth. Under normal condition, all green plants are autotrophs. Hence, they require the supply of inorganic materials from outside for synthesis of their own organic material. The source of inorganic compounds in plants is the minerals present in soil. As the source of these inorganic materials in the soil is minerals, they are called as mineral elements or mineral nutrients. Absorption, distribution and assimilation of inorganic compound or minerals by plants for synthesis of essential material for their growth, development, structure and physiology is called mineral nutrition. The mineral nutritional elements are taken up by plant roots from the soil solution in ionic form.

Following roles are ascribed to the mineral elements-

1. **Constituents of plant body:** Various mineral elements are constituents of the organic molecules found in the protoplasm and cell wall of plants. For example, the well known elements such as carbon, hydrogen, and oxygen are the components of carbohydrates that make up most of the cell wall and protoplasm. They are therefore called framework elements. Elements such as nitrogen, sulfur and phosphorus are required for the formation of protein, which is an important part of the protoplasm. (Nitrogen and sulfur are found in proteins, phosphorus in nucleoproteins and nucleic acids.) These elements are therefore called protoplasmic elements. Elements such as calcium (found in calcium pectate of the cell wall) and magnesium are important components of the cell wall and chlorophyll, respectively.
2. **Influence on the osmotic pressure of plant cells:** The cell sap contains mineral salts and organic compounds. The osmotic potential of plant cells depends on the concentration of mineral salts and organic compounds. Mineral salts dissolved in the cell sap partially influence the osmotic pressure of the cell. Adequate osmotic potential is required for water absorption and maintenance of cell turgidity.
3. **Catalytic Functions:** Certain minerals such as copper, zinc, iron, manganese, etc. act as catalysts in various enzymatic reactions occurring in plants.
4. **Influence on the pH:** The minerals taken up by the roots from the soil influence the H+ ion concentration and thus affect the pH value of the cell sap depending on the type of element, For example, sodium (Na+) and other monovalents increase membrane permeability while calcium (Ca++) and other divalents decrease it.
5. **Influence on the permeability of Cytoplasmic membranes:** The permeability of cytoplasmic membranes is influenced by cations and anions of the medium with which they are in contact. Some ions have an increasing effect on permeability, while others have a decreasing effect. For example, calcium and other divalent substances decrease membrane permeability, while sodium and some other monovalent substances increase it.
6. **Toxic effects of mineral elements:** Many mineral elements such as mercury, copper, arsenic, boron, lead, molybdenum, manganese, etc., in their ionic form, when present in higher than normal concentrations, have a toxic effect on the protoplasm.
7. **Antagonistic and Balancing effects:** Sometimes the effect of one ion can be reversed by another ion, which is called antagonism or balancing effects. For example, manganese at a concentration of 300 to 400 ppm on a dry weight basis in barley is toxic when the nutrient solution does not contain silicon, but harmless when silicon is present.

General roles of mineral elements in plants: When plant matter is burned in air, all organic components are destroyed, leaving a white residue called plant ash, which contains only the inorganic mineral elements in varying concentrations. Plant ash contains all essential and non-essential mineral elements except C, H, O, N and S, which are burned as gases.

Essential and Non-essential elements-

On the basis of their effects on plant, mineral elements are generally of two types-

(i) Essential
(ii) Non-essential

The term essential mineral element or mineral nutrient was proposed by Arnon and Stout (1939). They concluded three criteria must be met for an element to be considered essential. These criteria are: A plant must be unable to complete its life cycle in the absence of the mineral element, the element must be directly involved in plant metabolism, the function of the element must not be duplicated by another mineral element.

The non-mineral essential plant elements include hydrogen, carbon and oxygen. These are either taken up as a gas or water. Water culture and sand culture experiments had established that the elements- calcium, iron, sulphur, phosphorous, nitrogen, magnesium, potassium and magnesium were indispensable for the plants.

A list of essential nutrient elements is given below with their available forms and relative concentration in most of the higher plants (Table-1)

Table-1: Levels of essential elements required by most plants

Essential Element	**Chemical symbol**	**Available form**	**Concentration in dry matter (% or ppm)**
A. Micronutrients			
Molybdenum	Mo	Mo_4^{2-}	0.1
Nickel	Ni	Ni^{2+}	0.1
Copper	Cu	Cu^{2}+	6
Zinc	Zn	Zn^{2+}	20
Manganese	Mn	Mn2+	50
Boron	B	BO_3^{3-}	20
Iron	Fe	Fe^{2+}, Fe^{3+}	100
Chlorine	Cl	Cl^{-}	100
B. Macronutrients			
Sulphur	S	SO_4^{2-}, SO_2	0.1
Phosphorous	P	HPO_4^{-}, HPO_4^{2-}	0.2
Magnesium	Mg	Mg^{2+}	0.2
Calcium	Ca	Ca^{2+}	0.5
Potassium	K	K^{+}	1.0
Nitrogen	N	NO_3^{-} , NH_4^{+}	1.5
Oxygen	O	O_2, CO_2	45
Carbon	C	CO_2	45
Hydrogen	H	H_2O	6

***(Source:** Epstein 1972, 1999)*

2.3 MINERAL NUTRITION

Plants are autotrophs and they need inorganic nutrients to make organic compounds. They get these nutrients from the soil, air and water. The study of how plants obtain and use mineral nutrients is called mineral nutrition.

The term "fertility" refers to the inherent ability of soil to supply plants with nutrients in appropriate amounts and in appropriate proportions. The term "nutrition" refers to the interrelated steps through which an organism absorbs food, uses it for growth, and replaces tissues. Previously, plant growth was viewed in terms of soil fertility or how much fertilizer should be added to increase soil levels of mineral elements. Most fertilizers are formulated to address mineral deficiencies in the soil. The use of soilless mixtures, increased researches into nutrient culture and hydroponics, and advances in plant tissue analysis have led to a broader understanding of plant nutrition.

Plant nutrition refers to the interactions between mineral elements in soil or in a solution, as well as their role in plant growth. This relationship involves a delicate balance of important minerals that are essential for plant growth.

The term essential mineral element (or mineral nutrient) was proposed by Arnon and Stout (1939). They concluded three criteria must be met for an element to be considered essential. These criteria are:

1-A plant must be unable to complete its life cycle in the absence of the mineral element.

2-The function of the element must not be replaceable by another mineral element.

3- The element must be directly involved in plant metabolism.

Mineral Elements

It has been shown in numerous researches that certain elements are necessary for the healthy growth of plants. They are sometimes spoken of as essential elements and since some are needed in relatively large quantities and others in very small amounts, the former are referred to as “major” elements and the latter as “minor” or “trace” elements, or as micro nutrients.

Based on this Criteria Elements are Categorized in two heads:

- **Macronutrients –** These nutrients are present in large amount in the tissues of the plant. It includes Oxygen, Hydrogen, Nitrogen, Carbon, Phosphorus, Sulphur, Potassium, Magnesium and Calcium.
- **Micronutrients –** These are also called **Trace Elements** as these are required in very small amount. It includes Manganese, Iron, Zinc, Copper, Chloride, Nickel and Molybdenum.

2.3.1. Macronutrients

Every element participates in one or the other metabolic processes in the cells of plants and therefore, carries out several functions. The role of different macronutrient elements are given below-

1. Nitrogen: It is one of the very important nutrients required in greatest amount by the plants. It exists in soil in an organic form and is absorbed as No_3^- (Nitrate) and some are taken as No_2^- (Nitrite) or NH_4^+ (Ammonium). It is found in entire plant body, seeds and food storing regions in both organic and inorganic forms.

Functions of Nitrogen: Nitrogen is important for all parts of plants such as metabolically active cells and meristematic tissues. It is the major constituent of hormones, vitamins, nucleic acids and proteins. It increases the size of the leaves, promotes rapid growth along with fruit and seed development and hastens the maturity of the crop. Atmospheric nitrogen can be fixed and made available to the plant by certain bacteria. It is necessary for the formation of amino acids, proteins, DNA and RNA. It is essential for plant cell division and vital for plant growth.

Nitrogen deficiency: Nitrogen is very much mobile and is therefore, readily translocated to the younger leaves. Therefore, deficiency first appears on older leaves. Deficiency of nitrogen most often results in stunted growth, dormancy of lateral buds, late flowering, wrinkling of cereal grains inhibition of cell division, and chlorosis (leaves become yellow).

2. Sulphur: It is absorbed from the soil as sulphate ion or through the activity of micro-organisms by biological oxidation.

Functions of Sulphur: It is present in two amino acids and is the main constituent of several coenzymes like methionine and cysteine. Sulphur is also taken by leaves in gaseous form SO2. It is used in the formation of Sulphur- containing amino acids e.g., cysteine, cysteine and methionine. Sulphur is essential for the synthesis of Sulphur-containing vitamins, e.g., coenzymes, biotin and thiamine. It imparts flavor to many vegetables e.g., onion, garlic and mustard oil and increase growth, cell divisions and fruiting.

Sulphur deficiency symptoms: Sulphur deficiency results in a uniform pale green chlorosis throughout the plant that seems remarkably similar to nitrogen deficiency. With Sulphur depletion, problems tend to show up on the younger leaves first followed by the older leaves. Deficient plants are small and their growth is retarded, a general chlorosis, followed by the production of anthocyanin pigments in some species. The other effects of Sulphur deficiency are – hard woody stem and some accumulation of nitrates and carbohydrates, an extensive root system etc. Symptoms may vary between plant species.

3. Phosphorous: It is absorbed by the plants from the soil in the form of phosphate ions $H_2PO_4^-$ and HPO_4^{2-}.

Functions of Phosphorous: It is a constituent of cell membranes, nucleic acid and nucleotides, certain coenzymes of NAD, NADP and ATP ("energy unit" of plants forms during photosynthesis). It is involved in several key plant functions, including synthesis of nucleoproteins, energy transfer, transformation of sugars and starches, nutrient movement within the plant and transfer of genetic characteristics from one generation to the next.

Phosphorous deficiency symptoms: Phosphorous deficiency symptoms resemble with nitrogen but they are comparatively less developed. Similar to nitrogen, phosphorous deficiency may cause premature leaf fall and purple anthocyanin pigmentation. The anthocyanin is found in excessive amount. Plants show stunted growth due to abnormal cell-division. Phosphorous deficiency may create an imbalance in the storage of carbohydrates. Plants show stunted growth due to abnormal cell-division and develop a characteristic dark blue-green or purple colouration and brown necrotic areas are developed on leaves and petioles.

4. Calcium: Calcium is present in soil, in the form of cations or in mineral salts like anorthite ($Ca\text{-}Al_2\text{-}Si_2O_8$) and calcite ($CaCO_3$).

Functions of Calcium: It is taken up by roots from the soil solution and delivered to the shoot via the xylem. Calcium, in the form of calcium pectate, is responsible for holding together the cell walls of plants. In cell walls, calcium forms relatively insoluble salts by reacting with pectic acids in the middle lamella. Calcium pectases is essential for the continued growth of the apical meristems. It also activates specific plant enzymes, which send signals to the plant cells that coordinate certain growth activities. As a soil amendment, calcium helps to maintain chemical balance in the soil, reduces soil salinity, and improves water penetration, activate phospholipase, arginine kinase and ATP. It promotes translocation of carbo-hydrates, amino acids and root development.

Calcium deficiency symptoms: Sulphur is relatively immobile and therefore, deficiency symptoms appear first in young leaves. Organic acid metabolism is seriously disturbed due to the calcium deficiency. Its deficiency induces deformation of affected leaves, Necrosis at the tips and margins of young leaves, general chlorosis, highly branched, short, brown root systems, bulb and fruit abnormalities.

5. Potassium: It is found in soil solution in non-exchangeable or fixed form and in the cell in ionic form. It is found mostly in all cells except cork cells.

Functions of Potassium: Potassium is the only monovalent cation essential for plant growth. It helps in determining anion-cation balance and turgidity in cells and is involved in protein synthesis. Potassium is essential for the formation of sugar and starch and also for their translocation throughout the plant. It is also needed in cell division, reduction of nitrate, development of chlorophyll, opening and closing of stomata, etc. It maintains water balance and hydration of protoplasm and controls permeability of cytoplasm.

Deficiency symptoms of Potassium: Potassium deficiency inhibits synthesis of protein, which results in the accumulation of organic nitrogenous compounds in the plant cells. Scorching of older leaves on fruit trees is the most important symptom. The leaf tips and margins show general chlorosis. Potassium scarcity shows yellowing of leaves and the stem becomes thin. It also shows shorter internodes, loss of apical dominance, bushy habit, loss of cambial activity, plastid disintegration and increase in rate of respiration.

6. Magnesium: Magnesium is indispensable for the formation of chlorophyll, since it is one and the only mineral constituent of the chlorophyll molecule. Magnesium is present in the soil in water soluble, exchangeable and fixed forms. It is present in the soil solution in the form of magnesite ($MgCO_3$), dolomite ($MgCO_3.CaCO_3$), and Olivine [$(MgFe)_2$ SiO_4]. It is found in sufficient quantity in seeds of pulses and cereals.

Functions of Magnesium: It plays an important role in synthesis of ATP from ADP and inorganic phosphate. Magnesium acts as a activator of enzymes in phosphate transfer reactions in carbohydrates metabolism. It helps in fat synthesis and nucleoprotein synthesis. It is also believed to be an important binding agent in ribosomes where protein synthesis takes place. Magnesium probably combines strongly with ATP and makes it easy to break the bond.

Magnesium Deficiency Symptoms: Magnesium deficiency includes extensive interveinal chlorosis of the leaves. The older leaves are affected first and the younger leaves are affected later on. Ultimately leaves develop anthocyanin pigments and necrotic spots or purple spots on mature leaves.

2.3.2 Micronutrients or Trace Elements

The role of the micronutrient elements are given below-

1. Manganese: It is absorbed largely as bivalent manganous ions. It is found in the plant ash especially in the leaves.

Functions of Manganese: Manganese functions as an enzyme activator in several reactions of respiration and nitrogen metabolism. The enzymes-malic dehydrogenase and oxalosuccinic decarboxylase in respiration and nitrite reductase and hydroxylamine reductase in nitrogen metabolism require manganese as activator. Manganese helps in splitting of water to liberate oxygen during photosynthesis.

Deficiency Symptoms of Manganese: Manganese deficiency causes necrotic and chlorotic spots in the interveinal areas of the leaf. Its deficiency also has a marked effect on chloroplasts which lose chlorophyll and starch grains, turn yellow-green in colour. They become vacuolated and granular and finally disintegrate. Manganese deficiency causes Grey speck disease in oats.

2. Iron: It is absorbed in the both the ferrous (Fe^{++}) and ferric (Fe^{+++}) forms. It is in the insoluble form in a neutral or alkaline soil and always present in the soluble form in an acidic soil and is therefore readily absorbed by the plants. This element is always found to be present in the chromatin material of nucleus and protoplasm.

Functions of Iron: Iron is important constituent of Ferredoxin which plays important role in primary photochemical reactions and Biological Nitrogen fixation. Iron is also an important constituent of iron porphyrin proteins like cytochrome catalases and cytochrome peroxidases.

Deficiency of Symptoms: Deficiency of iron causes interveinal chlorosis of leaves. Iron deficiency also induces chloroplast disintegration, death of root tips, decrease in respiratory rate, etc.

3. Boron: Boron is largely absorbed from soil in the form of soluble borate and tetraborate ions. It is present in the soil in the form of boric acid, calcium and manganese borate and silicates.

Functions of Boron: Boron probably facilitates the translocation of sugars. It plays an important role in cellular differentiation and development of fertilization, fat metabolism, hormone metabolism, active salt absorption, photosynthesis etc. Boron helps in the formation of the nodules of the leguminous plants.

Deficiency Symptoms of Boron: Boron deficiency results in the death of shoot tip, stunted root growth, suppressed formation of flowers. The leaves have coppery texture. Accumulates carbohydrates and amino acids in leaves. Physiological diseases like internal cork formation in apples, top root of tobacco, cracked stem of celery, browning of cauliflower, heart root of sugar beet, rolling of leaves in potatoes are development as a result of boron deficiency.

4. Copper: Copper is largely absorbed by plants as cupric or cuprous ions. In soil, it is mainly present as chalcopyrite ($CuFeS_2$) and copper sulphide.

Functions of Copper: The element is required in very small quantity. Higher concentration of copper is toxic to plants. Copper undergoes alternate oxidation and reduction as its acts as an electron carrier and part of certain enzymes like phenolases, laccase and ascorbic acid oxidases. It is a part of plastocyanin and thus may function in electron transport chain of photosynthesis.

Copper deficiency Symptoms: Copper deficiency causes necrosis of the tip of the young leaves. It also causes exanthema disease (formation of deep slit in bark from which gum exudes) in some trees.

5. Zinc: Plants use zinc in the form of Zn++ which are absorbed to the soil surface and no organic matters in exchangeable form. It is usually found in the seeds.

Functions of Zinc: Zinc helps in the formation of chloroplasts. Zinc functions as activator of certain enzymes, e.g., carbonic anhydrase, alcohol dehydrogenase, hexose, kinase, etc. It is also involved in biosynthesis of the growth hormone like auxin, indole-3-acetic acid (IAA). It also plays an important role in protein synthesis.

Zinc Deficiency Symptoms: Zinc deficiency causes reduced stem growth due to decreased synthesis of auxin. It results in stunted vegetative growth and distorted leaves. Chlorosis of the

older leaves which starts from tips and margins. The internodes are reduced in size and the effect in sometimes referred to as 'little leaf' diseases. Zinc deficiency causes mottle leaf disease in apple, citrus, walnut etc., Khaira disease in rice. In maize, zinc deficiency produces 'white bud disease' in which flowering and fruit formation is reduced.

6. Molybdenum: Molybdenum exists to a large extent in soils in the form of molybdate ion and is active in hexavalent state. In exchangeable form it is found absorbed on soil particles while in non-exchangeable form it is present as constituent of soil mineral and organic matter. Molybdenum is required in very little quantity by the plants.

Functions of Molybdenum: Molybdenum plays important in the nitrogen metabolism and gaseous nitrogen fixation. It acts as an activator for the enzyme nitrate reductase.

Molybdenum Deficiency Symptoms: Molybdenum deficiency causes chlorotic interveinal mottling of lower leaves. It is followed by marginal necrosis and infolding of leaves. Flower formation is inhibited. 'Whiptail' disease of cauliflower has been reported to be due to deficiency of the molybdenum.

7. Chlorine: Chlorine is absorbed from the soil solution as chloride ions (Cl^-)

Functions of Chlorine: It helps in maintaining anion-cation balance in cells and is essential for oxygen evolution in photosynthesis. It is required for cell division in roots and leaves.

Chlorine Deficiency Symptoms: Chlorine deficiency in plants causes wilting of leaves which may eventually attain a bronze colour and stunted roots thickened near the tips and also reduced fruiting.

8. Nickel: Ni is an essential mineral for the urease enzyme which breaks down urea into usable forms of nitrogen. It is believed to be important for mobilization of nitrogen during seed germination. In addition, this element is required for the microbes for its *hydrogenase* enzyme. It is also essential for iron uptake. Seeds will not germinate without Ni.

Deficiency Symptoms: Nickel deficiency in plants causes accumulation of urea in leaves, which results necrosis of leaf tips.

Role of other Elements

Many other elements are present in plants in large or small amounts. It is assumed that most of these non-essential elements are quite important for the normal growth and development of the plants. Some of the most common are sodium, cobalt, selenium, silicon, Gallium, vanadium, etc.

Sodium: It is essential for blue green algae and at least for some of the higher plants. It regulates the transport of amino acids to the nucleus and therefore, controls the synthesis of nucleoprotein. It is believed to be involved in stomatal opening, oxalic acid accumulation, nitrate reductase activity, crassulacean acid metabolism, Hatch-Slack cycle and in maintenance of water balance.

Cobalt: It is part of vitamin B12 which is itself a part of an enzyme. It is known to be essential in symbiotic organisms for nitrogen fixation. It participates in leghemoglobin metabolism. It is needed by the enzyme ribonucleotide reductase in Rhizobium.

Selenium: Certain plants like Astragalus are believed to have selenium metabolism. It some plants in behaves like sulphur.

Silicon: It is essential for the formation of the wall of diatoms. It reduces transpiration and improves resistance to pathogens. It neutralizes phosphate deficiency and reduces the toxicity caused by iron and manganese.

Aluminum: It has been reported to stimulate plant growth in isolated instances.

Gallium: It is needed by the fungus *Aspergillus niger* in traces.

Vanadium: It is needed by Scenedesmus in traces.

According to Mengel & Kirkby (2001), the essential elements have been divided into four basic groups.

1. Nitrogen and sulfur form the first group of essential elements. Plants absorb these nutrients through biochemical reactions that involve oxidation and reduction to form covalent bonds with carbon and create organic compounds.
2. The second group is important in energy storage reactions or in maintaining structural integrity.
3. The third group is present in plant tissue either as free ions dissolved in the plant water or as ions electrostatically bound to substances such as the pectic acids present in the plant cell wall. Elements in this group play important roles as enzyme cofactors and in the regulation of osmotic potentials.
4. The fourth group, which includes metals such as iron, plays an important role in electron-transfer reactions.

Table-2: Classification of Plant mineral nutrients according to biochemical function

Mineral nutrient	Functions
Group 1	**Nutrients that are part of carbon compounds**
N	Constituent of amino acids, amides, proteins, nucleic acids, nucleotides, coenzymes, hexosamines, etc.
S	Component of cysteine, cystine, methionine. Constituent of lipoic acid, coenzyme A, thiamine pyrophosphate, glutathione, biotin, 5'- adenylsulfate, and 3'-phosphoadenosine.
Group 2	**Nutrients that are important in energy storage or structural integrity**
P	Component of sugar phosphates, nucleic acids, nucleotides, coenzymes,

	phospholipids, phytic acid, etc. Has a key role in reactions that involve ATP.
Si	Deposited as amorphous silica in cell walls. Contributes to cell wall mechanical properties, including rigidity and elasticity.
B	Complexes with mannitol, mannan, polymannuronic acids, and other constituents of cell walls. Involved in cell elongation and nucleic acid metabolism.
Group 3	**Nutrients that remain in ionic form**
K	Required as a cofactor for more than 40 enzymes. Principal cation in establishing cell turgor and maintaining cell electroneutrality.
Ca	Constituent of the middle lamella of cell walls. Required as a cofactor by some enzymes involved in the hydrolysis of ATP and phospholipids. Acts as a second messenger in metabolic regulation.
Mg	Required by many enzymes involved in phosphate transfer. Constituent of the chlorophyll molecule.
Cl	Required for the photosynthetic reactions involved in O_2 evolution.
Mn	Required for activity of some *dehydrogenases, decarboxylases, kinases, oxidases,* and *peroxidases*. Involved with other cation- activated enzymes and photosynthetic O_2 evolution.
Na	Involved with the regeneration of phospho enolpyruvate in C_4 and CAM plants. Substitutes for potassium in some reactions.
Group 4	**Nutrients that are involved in redox reactions**
Fe	Constituent of cytochromes and nonheme iron proteins involved in photosynthesis, N_2 fixation, and respiration.
Zn	Constituent of *alcohol dehydrogenase*, *glutamic dehydrogenase, carbonic anhydrase*, etc.
Cu	Component of *ascorbic acid oxidase, tyrosinase, monoamine oxidase, uricase, cytochrome oxidase, phenolase, laccase*, and plastocyanin.
Ni	Constituent of *urease*. In N_2-fixing bacteria, constituent of *hydrogenases*.
Mo	Constituent of *nitrogenase*, *nitrate reductase*, and *xanthine dehydrogenase*.

(*Source*: After Evans and Sorger 1966 and Mengel and Kirkby 2001)

2.4 TECHNIQUES TO STUDY THE ROLE OF MINERAL NUTRIENTS

In general, soil supplies the mineral nutrients for plant growth. The various elements that have entered into the composition of the plant body can be determined by ash analysis, and those essentially required by the plant by solution culture experiments.

1. Ash analysis

The plant is heated to around 6000 degrees Celsius, and then its ash content is analyzed. The ash consists only of mineral elements, which are all released in the form of gases during

decomposition. The primary elements, carbon, hydrogen and oxygen, are emitted as CO2, water vapor and oxygen and therefore cannot be detected by this method. Nitrogen also cannot be accurately detected by this method, as some nitrogen is released as ammonia or nitrogen gas. . By analyzing the ash left behind after a plant has been burned, the presence of other minerals can be detected. Detection of mineral elements can be done either by wet digestion or by ash analysis of oven-dried powdered plant material. The ash is dissolved in warm diluted HCl or HNO3 and used to detect and quantify the elements present, including modern techniques such as atomic emission spectroscopy, X-ray emission spectroscopy and ion chromatography, subject to various physical, chemical, or physicochemical methods.

2. Solution culture experiments

The essentiality of a specific mineral element for the normal growth and development of the plant are often determined by the solution culture method. A well-defined nutrient medium is used to identify the elements essential to plant growth and to record the symptoms produced by their absence or deficiency. In the solution culture method, the roots of the plant are placed in liquid nutrient solution. The solution containing those essential minerals that the plant absorbs from the soil through its roots is called a nutrient solution. When all the mineral elements are present in a solution, it is said to be a normal nutrient solution, and when one of the essential elements in the nutrient solution is not added, it is said to be a deficient nutrient solution. If we want to study the importance of a mineral, it is not added to the nutrient solution. It means that the solution is a deficient nutrient solution for that particular element. Now the plants are grown in this deficiency solution and compared with those in the normal solution. If there are deviations from normal in the morphological, anatomical and physiological characteristics of plants, these deviations are due to a lack of this element. Through this method we can find out the importance and functions of different elements.

Seedlings are grown in thoroughly washed glass, glazed porcelain or plastic containers. Their roots are placed in a carefully made up nutrient solution. Only pure salts and glass distilled water should be used for preparing nutrient solution and care should be taken to exclude organic matter, microbial contaminants and dust. It is better if the solution is properly aerated with the help of a small aquarium pump.

Hydroponics (Soilless growth)

The technique of growing plants without soil is known as hydroponics. The word hydroponics comes from two Greek words "*hydro"* meaning water and "*ponos"* meaning labor. This word was first used by Dr William Frederick Gericke in 1937.

In 1860, Von Sachs, a German botanist demonstrated that plants grow without soil if they are provided with a nutrient-rich solution and fulfil their oxygen demand. The term "hydroponics" is now being used to describe plants that are rooted in sand, gravel, or other similar material. This is done by soaking the material with a recycling flow of nutrient-rich water. Hydroponics also called aquaculture, nutriculture, soilless culture, or tank farming (Fig. 2.1).

Hydroponics is a type of Horticulture and subset of Hydro culture which is a method of growing plant, usually crops without soil by using mineral nutrients solution in an aqueous solvent. Hydroponics removes the barriers between the plant and its nutrients. This provides the roots with access to water, oxygen and nutrients that it needs to grow and survive.

Hydroponics system is a method of the cultivation of plants in nutrient-enriched water, with or without the mechanical support of an inert medium. Here soil is substituted by sterile mediums such as clay, sand, gravel, pellets and perlite to give stability to roots.

Nutrients are passed through roots differently, based on the type of hydroponic system used, oxygen is pumped through, pH level is regulated and sufficient light is provided to carry out photosynthesis. In the areas where natural light is not available, artificial lighting is determing the deficiency symptoms of various nutrients in plants and to find out essential nutrients for the plants growth and development.

The plants are supported by wire netting and cultivated in huge tanks filled with nutritional solution. The tanks are situated in controlled environments in green houses and have a system for controlling and pumping the solution. When the plants flower after about a month in the greenhouse, they are further supported by strings attached to the greenhouse ceiling. Tank farming is another name for this method of soilless cultivation because the plants are grown in huge tanks.

The main advantage of using hydroponics method is:

- The equipment can be made automatic, avoiding the labour and expense of watering the plants.
- There is generally uniformity of plant growth and plant products.
- Soil-borne diseases and pests are eliminated.
- It allows for a much quicker growth rate, it requires very less space.
- Absolute control over the nutrients as required by the plants.
- Plant growth is completely dependent on the nutrient solution provided. Thus, there is controlled plant growth.
- It is possible to provide whatever nutrient environment is desirable.
- The acid-base balance (pH) of the nutrient solution can be easily set and maintained.
- Because there is no soil, there is also no need for harmful pesticides or chemicals. There’s also a lower risk of plant disease or exposure to external elements.
- Complete control over the temperature, humidity levels and the intensity of light.
- Water present in the system can be reused, which facilitates water conservation.
- Improved yield and growth.

The disadvantages associated with hydroponics is-

- As compared with field production, production by hydroponics is limited.
- Initial expenses are high.
- It is a time-consuming process.
- Some water borne diseases can spread rapidly in recirculation system.
- Experiences and technical knowledge is required.
- Requires constant monitoring and maintenance.
- Vulnerable to power outages.

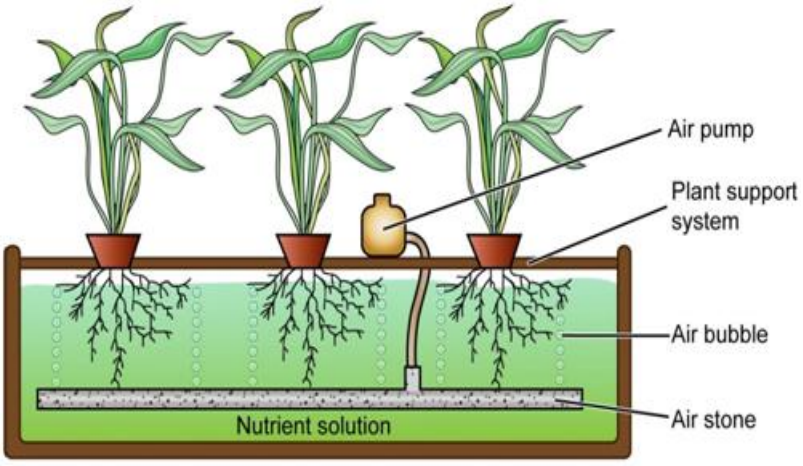

Fig. 2.1: Hydroponic growth system (Based on Taiz et al)

Hydroponics is used in the commercial production of many greenhouse and indoor crops, such as tomato *(Solanum lycopersicum)*, cucumber *(Cucumis sativus)*, and hemp *(Canabis sativa)*.

There are different types of hydroponic systems which include the following:

1. Nutrient Film techniques
2. Aeroponic systems
3. Ebb and Flow (Drain and flow)
4. Drip
5. Wick System
6. Water Culture

1- Nutrient Film techniques: NFT is a hydroponic growing system where the term 'nutrient film' refers to the constant shallow stream of nutrient solution flowing across the bottom of the plant's roots. In this system, the roots of the plants are suspended in slightly slopped channels, so-called gullies. A thin, film-like, shallow stream of recirculating water is pumped from a reservoir to the top of the channel, and falling water is collected and reused. The water should be shallow enough that only the bottom of the roots are submerged while the upper roots will

remain dry to ensure the plants can oxygenate. The NFT system is ideal for short-term crops such as lettuce, leafy crops and herbs **(Fig. 2.2).**

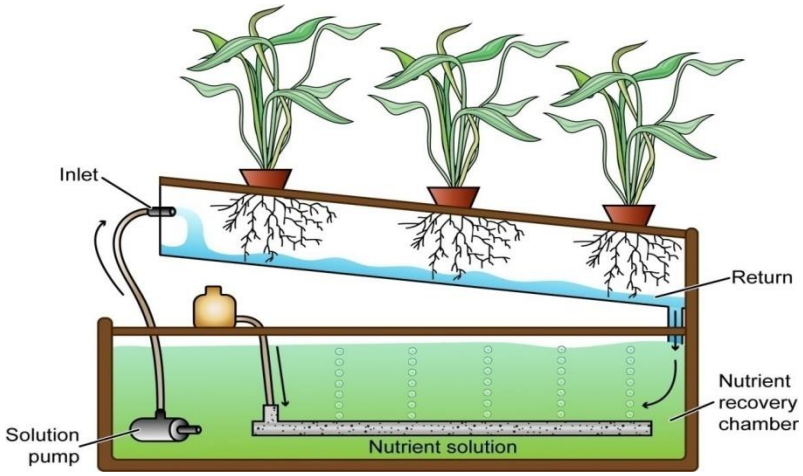

Figure. 2.2: A Nutrient film growth System (Based on Taiz et al)

2- Aeroponics system: In this technique, rooted plants are placed in a special type of box, with their shoots exposed to the air and the roots lying inside the box. The nutrient solution is stored in the bottom of the box. Then the nutrient mist (a cloud of moisture in the air) is sprayed onto the roots by a motor-driven rotor. This system is also used for research purposes in the laboratories **(Fig.2.3).**

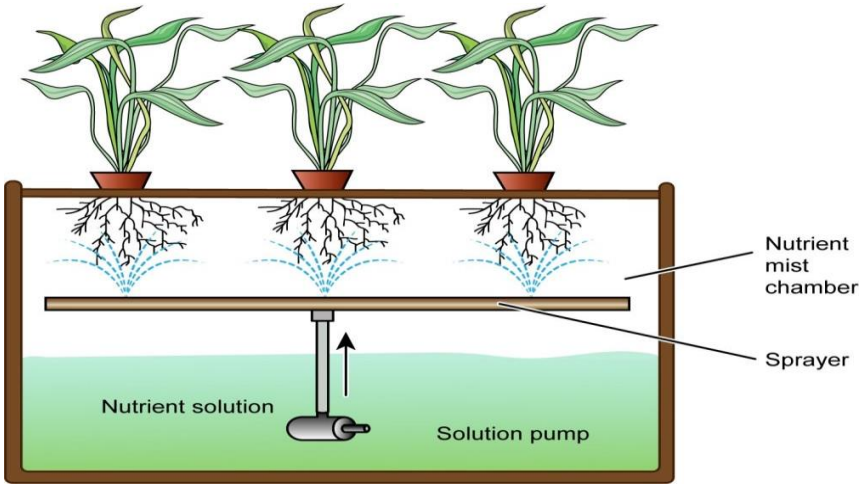

Fig. 2.3: An Aeroponic Growth System (Based on Taiz et al)

3- Ebb and Flow system: The nutrient solution rises at intervals to submerge plant roots and then pull back, exposing the roots to humid atmospheric conditions. It requires higher concentrations of nutrients to sustain plant growth. (Fig.2.4)

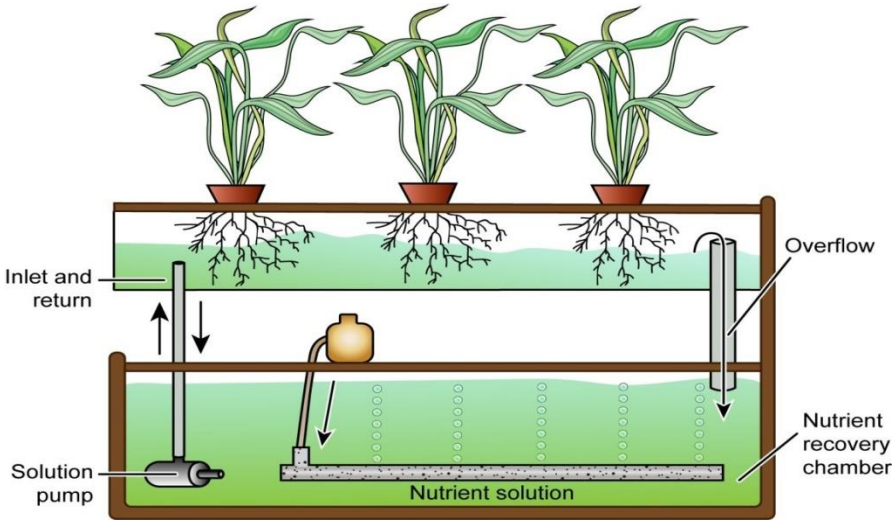

Fig.2.4: An Ebb and Flow system ((Based on Taiz et al)

4- Drip System: In the drip system, the water-based nutrient solution is delivered to the root system of the plants through drip irrigation. The drip system supplies each plant directly with water-based nutrient solution. The solution is delivered through a network of valves, emitters, hoses and tubes. This type of low-flow irrigation is very water efficient and avoids evaporative waste by providing moisture through slow dripping at the base of the plants, rather than mimicking rain from above. (Fig.2.5)

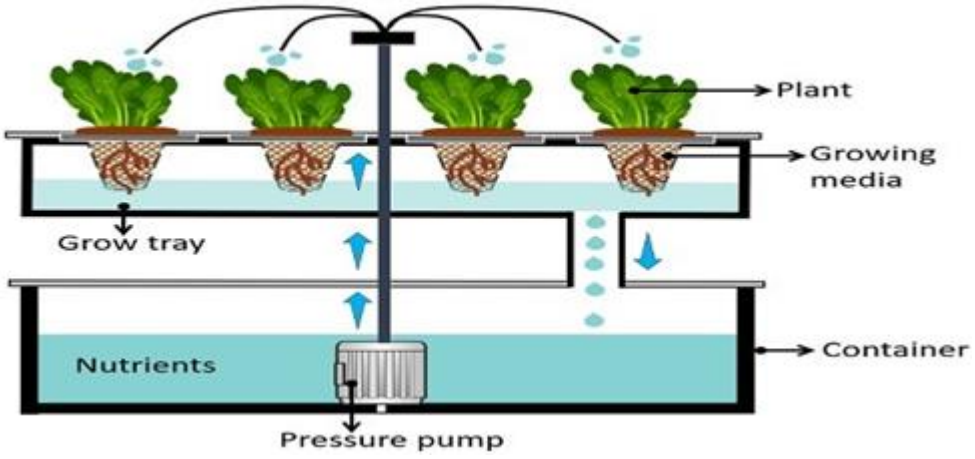

Fig. 2.5: Drip system (Source- Google images)

5-Wick System: In a wick system, the nutrients and water are transported to the plant roots via a wick, such as a rope or piece of felt. The plants are suspended in a sort of grow tank, which is a reservoir for water and nutrient solution. One end of the wick is in the solution and the other end

of the wick is in the growing medium. This allows the wick to transport the water and nutrients at the same rate that the plant roots need the nutrients. Whenever the roots are ready to absorb, they absorb the nutrients from the wick. Wick systems do not require air or water pumps. It is ideal for smaller plants. (Fig.2.6)

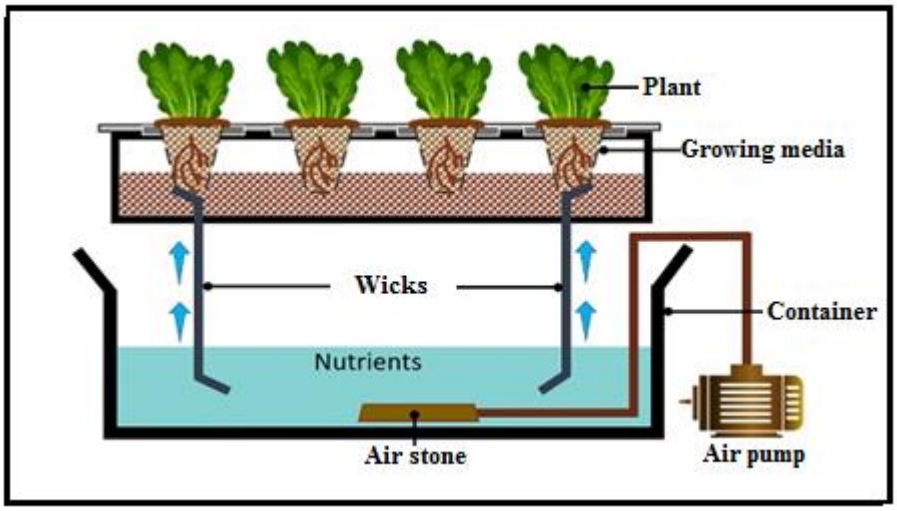

Fig.2.6: Wick System (Source- Google images)

6-Deep Water Culture (DWC): Deep water growing, or direct water culture, is a type of hydroponic growing method in which the roots of the plants are continuously suspended in nutrient-rich, highly oxidized water. Oxygen is pumped into the reservoir by an air pump and then pushed through an air stone. The oxygen allows the plant to absorb the maximum amount of nutrients, resulting in accelerated plant growth. Once the plant has established a robust root system, the amount of water in the reservoir, often a bucket, is lowered. (Fig.2.7)

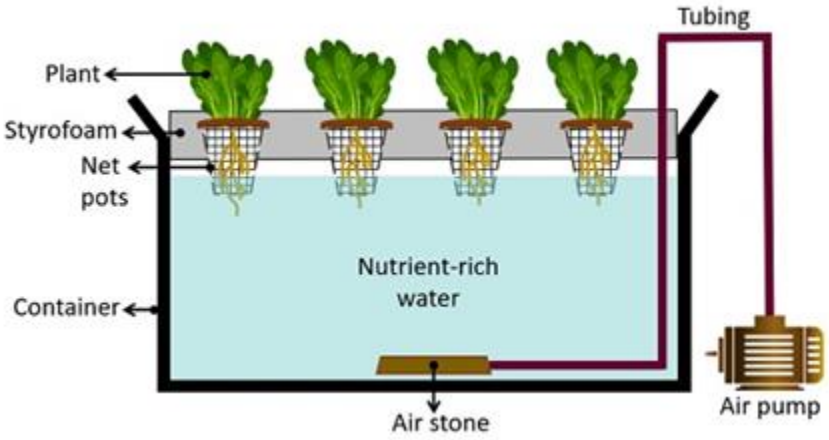

Fig.2.7: Deep water culture system (Source- Google images)

2.6 SUMMARY

Plants are autotrophic organisms capable of using the energy from sunlight to synthesize all their components from carbon dioxide, water, and mineral elements. Although mineral nutrients continually cycle through all organisms, they enter the biosphere predominantly through the root systems of plants. After being absorbed by the roots, the mineral elements are translocated to the various parts of the plant, where they serve in numerous biological functions. The process in which the absorption and utilization of various mineral ions by plants for their growth and development is called mineral nutrition. Studies of plant nutrition have shown that specific mineral elements are essential for plant life. These elements are classified as macronutrients or micronutrients, depending on the relative amounts found in plant tissue. The elements which are necessary for normal growth and development of the plant are said to be macronutrients. Potassium, calcium, magnesium, carbon, hydrogen, oxygen, nitrogen, Sulphur and phosphorous belongs to this category. There are other elements which are specifically required by the plant in very minute quantities and these elements are known as micronutrient. Manganese, copper, molybdenum, iron, zinc, boron and nickel are essential micronutrient. The elements required by the plants become the essential constituents of protein, carbohydrate, fat, nucleic acid etc., and take part in various metabolic processes.

Certain visual symptoms are diagnostic for deficiencies in specific nutrients in higher plants. Nutritional disorders occur because nutrients have key roles in plants. They serve as components of organic compounds, in energy storage, in plant structures, as enzyme cofactors, and in electron transfer reactions. Mineral nutrition can be studied through the use of solution culture, which allows the characterization of specific nutrient requirements. Soil and plant tissue analysis can provide information on the nutritional status of the plant-soil system and can suggest corrective actions to avoid deficiencies or toxicities.

There are some techniques to study the role of mineral nutrients. Ash analysis is a technique in which the existence of inorganic elements in plants can be detected by the chemical tests of plant ash. **Another technique of growing plants in nutrient-enriched water without soil is called as soilless growth or hydroponics.** There are different types of hydroponic systems such as- Nutrient Film technique, Aeroponic systems, Ebb and Flow, Drip, Wick System and Deep Water Culture.

2.7 GLOSSARY

Aeroponics growth System: In this system, a high-pressure sprayer pump is used to sprayer pump is used to spray continuously nutrients solution on plant roots enclosed in a tank.

Ebb-and Flow system: In this system; a solution pump periodically fills the upper chamber with nutrient solution and then drains the solution back into the main tank.

Essential elements: Elements those are indispensable or necessary for the normal growth and development of plants.
Macronutrients: It is generally present in plant tissue in large amounts (in concentration of 1 to 10 mg per gm of dry matter).
Micronutrients: Micronutrients or trace elements are needed in very small amounts (i.e., equal to or less than 0.1 mg per gm of dry matter.
Hydroponics: The practice of growing plants in nutrient enriched water without soil.
Nutrient solution: A solution containing only inorganic salts for growth of plants in sunlight without soil or organic matter.
Nutrient film growth system: A form of hydroponic culture in which the plant roots lie on the surface of a trough, and the nutrient solution flows over the roots in a thin layer along the trough.

2.8 SELF ASSESSMENT QUESTION

2.8.1 Multiple choice Questions:

1. The micronutrient among these is
(a) Zn (b) N
(c) P (d) Ca

2. The yellowing of leaves, called chlorosis, is usually caused by the deficiency of-
(a) Sodium (b) phosphorous
(c)Magnesium (d) Calcium

3. Chlorosis is:
(a) yellowing of leaves due to the absence of chlorophyll
(b) yellowing of leaves due to low chlorophyll contents
(c) greening of leaves due to high contents of chlorophyll
(d) yellowing of leaves due to high contents of nitrogen

4. Mottle leaf in citrus plants is due to the deficiency of:
(a) boron (b) zinc
(c) magnesium (d) molybdenum

5. The deficiency of which of the following elements may cause leaf tip bending:
(a) Sulphur (b) phosphorus
(c) calcium (d) nitrogen

6. 'Khaira' disease of rice is developed due to the deficiency of:
(a) copper (b) sodium

(c) zinc (d) molybdenum

7. The essential nutrient element required by plants in the least quantity is:
(a) Chlorine (b) Zinc
(c) Molybdenum (d) Manganese

8. Which of these is not a nutrient for plants:
(a) water (b) mineral ions
(c) carbon dioxide (d) nitrogen gas

9. Death of stem and root tips occurs due to deficiency of:
(a) Calcium (b) Nitrogen
(c) Carbon (d) Phosphorus

10. Premature leaf fall occurs due to deficiency of:
(a) Calcium (b) Iron
(c) Phosphorous (d) Sulphur

11. The practice of growing plants in nutrient enriched water without soil is called as-
(a) solution culture (b) hydroponics
(c) aeroponics (d) none of the above

12. In aeroponics, the plants roots are suspended-
(a) freely in air (b) in a nutrients mist chamber
(c) in thin film of nutrient solution (d) none of the above

2.8.2 True or False:

1. The symptoms of magnesium deficiency appear first in the mature leaves.
2. Deficiency of molybdenum causes whiptail disease of cauliflower.
3. Zinc is responsible for causing die-back disease in plants.
4. Boron deficiency may cause leaf tip bending.
5. A plant requires potassium mainly for opening and closing of stomata.

2.8.1 Answer Key: 1-(a), 2-(c), 3-(b), 4-(b), 5-(d), 6-(c), 7-(c), 8-(d), 9-(a), 10-(c), 11-(b), 12-(b)

2.8.2 Answer Key: 1-True, 2- True, 3-False, 4-False, 5- True

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2.11 TERMINAL QUESTIONS

2.11.1 Short answer type questions:

1. Define the following: (a) Micronutrients, (b) Macronutrients
2. Mention the symptoms of any four mineral deficiencies in plants.
3. Differentiate between essential and non-essential mineral elements.
4. State deficiency symptoms of Manganese, Iron, Boron, and Zinc.
5. Why do plants need potassium and magnesium.
6. Discuss hydroponics system.
7. Short note on:
   (a) Aeroponics
   (b) Nutrient Film Technique

2.11.2 Long answer type questions:

1. Write an essay on mineral nutriation in plants.
2. List the macronutrients and mention their major functions.
3. What are traces elements? What symptoms are caused by their deficiency?
4. Make a list of macronutrients and mention their major functions.
5. Write about any three macronutrients with their symptoms and deficiencies.
6. Describe any four micronutrients with their deficiencies.
7. What are essential elements in plant nutrition? Explain the role of any three of them.
8. Describe the techniques to study the role of Mineral Nutrients.

UNIT-3- MINERAL TRANSPORT

Contents:

3.1 OBJECTIVES

After reading this section you will know-

1. How the minerals are transported across the membrane
2. Active and passive transport methods
3. Types of passive transport methods like diffusion and facilitated diffusion
4. Ion channels and their role
5. Primary active transport and secondary active transport methods
6. Nernst equation and its importance
7. Anatomy of phloem tissue and mechanism of phloem transport
8. Munch hypothesis
9. Factors affecting translocation

3.2 INTRODUCTION

We know from previous chapter that the actual absorption of salts by roots is both passive and active. The mineral substances which are accumulated in the cytoplasm of root hair cells and in cortical cells are transported towards the xylem vessels that are radial transport. This transport is very specific, highly regulated and takes place through the symplast and apoplast. The transport of ions through the apoplast is based on the process of diffusion of ions at the cell wall level. The symplastic path is always active and occurs from cell to cell through the plasmadesmata or through across the membrane. Transport of the minerals from the roots towards the leaves and other plant parts is performed through the xylem along with the transpiration stream.This movement inside the xylem is a passive process. The distribution of mineral ions is determined by the functional activity of the tissue. The more share is delivered to the young growing tissues.

3.3. TRANSFER ACROSS THE MEMBRANE

The peculiar structure and chemical composition of the biological membranes make them semi-permeable in nature allowing the transport of some substances across them which are very small in size or hydrophobic in nature and impermeable for the others especially large hydrophilic and charged molecules. Cellular membranes also contain certain transport proteins which help the transfer of selected ions and other molecules. Most of the transport proteins have specificity for their transport molecules allowing them to cross the membrane, passing through the transport proteins. There are three main groups of the transport proteins viz. channels, carriers and pumps. Based on the utilization of metabolic energy during transfer of the molecules the transfer across the membrane may be passive or active or both.

3.4 PASSIVE AND ACTIVE TRANSPORT

The mineral salts are present in the soil solution in dissociated ion condition and are absorbed by the root hairs and then translocated through xylem upstream to other parts of the plant. Based on utilization of metabolic energy of the cell during the transportation process there are two major mechanism for mineral transport – 1. Passive transport 2. Active transport

3.4.1 Passive Transport

Passive transport of the ions involves the process of simple diffusion, facilitated diffusionand transport of ions through ion channels etc. In this transport process, there is no utilization of the metabolic energy by the cell. The transport process occurs along the concentration gradient of the substance i.e. from higher concentration to the lower concentration and also down to their electro-chemical gradient. Facilitated diffusion and transport through the ion channels are also known as mediated transport because it requires the presence of transport membrane proteins which span the plasma membrane and help to cross the membrane through their inner hollow core.

3.4.1.1 Simple Diffusion

It is a non-mediated diffusion in which certain small, non-polar molecules like oxygen, carbon dioxide and ethanol move across the membrane by process of simple diffusion. The movement of molecules is from the region of higher concentration to the lower concentration i.e. along concentration gradient. The diffusion of one substance is independent of the diffusion of the other substance. The rate of diffusion of gases is greater than the rate of diffusion of liquids and rate of diffusion of liquids is more than the rate of diffusion of solids. Movement of molecules across the membrane is by simple diffusion is a slow process of transportation.

3.4.1.2 Facilitated Diffusion

In facilitated diffusion, specific molecules move from higher to lower concentration only with the help of transport proteins that mediate the movement of solute molecules across the membrane. These proteins are called permeases or transporters. These proteins are integral proteins that contain several trans membrane segments and therefore traverse the membrane multiple times. Transporter proteins from hydrophilic channels through the membrane that allow the passage of solutes through its core. These proteins bind their transporting molecules like binding of enzymes to its substrate through several weak non-covalent bonds, which results into negative free energy change and thus facilitate the transport of molecule.

3.4.1.3 Ion Channels

The transport of many ions is accelerated by the presence of the specific transport proteins called ion channels present in the plasma membrane. These proteins bind with their specific substrate ions, may or may not change their configuration after binding the ion and change the position of ion from one side of the membrane to another side and thus facilitate the transport of the specific ion. The movement of ions, in mediated transport way is according to the concentration gradient of the ion and without utilization of the metabolic energy. The ion channels are remarkably specific. Most allow passage of only one kind of ion, so separate channels are needed for transporting ions like Na^+, K^+, Ca^{2+}, Cl^- etc. Ion channels may be cation channels which are very specific while the anion channels allow a wide range of anions including Cl^-, NO^-_3 and organic acids. These channels are bidirectional; they allow the passage of ions in both the directions. Most ion channels are gated, i.e. they can be open or close by conformational changes in protein, thereby regulating the flow of ions. They may be a. Voltage gated: These channels open and close in response to changes in membrane potential. Eg. $Na^+$and K^+ ion channels.

b. Ligand gated: These channels open and close by binding of specific substances to the channel proteins like binding of secondary messenger molecules like c-AMP.

c. Mechano-sensitive: These channels open and close in response to mechanical forces that act on membrane like in *Mimosa* leaves.

3.4.2 Active Transport

The active uptake of the ions in roots requires the utilization of metabolic energy of the plant and it occurs against the concentration gradient of the ion. The active transport is thermodynamically unstable i.e. endergonic and takes place only when coupled to an exergonic process such as absorption of sunlight, an oxidation reaction, hydrolysis of ATP and co-transport of some other molecule down to its concentration gradient. The rate and amount of the mineral uptake by this method is directly related with the expenditure of the metabolic energy. Mechanism of active transport can be understood by the Nernst Equation. The active ion absorption could be primary or secondary or both.

3.4.2.1 Primary Active Transport

The primary active transport of mineral ions is linked with utilization of metabolic energy other than the electrochemical potential gradient like energy stored in ATP, an oxidation reduction reaction. This energy is utilized by the special complex protein pumps, which are specialized primary active transporter structures. These pumps may be either electrogenic or electroneutral. The electrogenic ion transporter pumps involve the net movement of charge across the membrane while the electroneutral transport implies no net movement of the charge across the membrane. Eg. Na^+/K^+ ATPase of animal cell after ATP hydrolysis, pumps three Na^+ ions out for every two K^+ ions in, resulting in movement of one positive charge in each cycle. This is an example of electrogenic ion pump. While the H^+/K^+ ATPase pump, pumps one H^+ ion out of the

cell for every, one K^+ ion, after ATP hydrolysis, so there is no net movement of charge across the membrane, hence, it is an electro neutral pump. The movement of ions is always against their concentration gradient i.e. from lower concentration side to the higher concentration of the ions.

3.4.2.2 Secondary Active Transport

The secondary active transport of ions utilized the energy stored in electro-chemical potential gradient. It depends on the co-transport of two solutes, with the movement of one solute down its gradient driving the movement of other solute up its gradient. In plants, the uptake of specific mineral molecule depends on an electrochemical proton gradient as the driving force for secondary active absorption. The transport process is either symport or antiport depending on whether the two solutes move in the same or opposite directions. When two solutes are transported simultaneously and their transport is coupled, the process is called co-transport.

Symport and Antiport

In co-transport, the process is called symport, if the two solutes are moved in the same direction or antiport if the two solutes are moved in opposite directions. The transport protein involved in symport or antiport are called symporter and antiporter respectively as K^+/Na^+ ion symporters and Na^+/H^+ antiporter. In antiport movement, the energetically downhill movement of protons drives the active transport of a solute in opposite direction. In both types of secondary active transport, the transport of both the ions (one H^+ and another any specific ion or solute) is simultaneous with the protons moving against its concentration gradient and the energy driving this transport is provided by the proton motive force (Fig. 3.1).

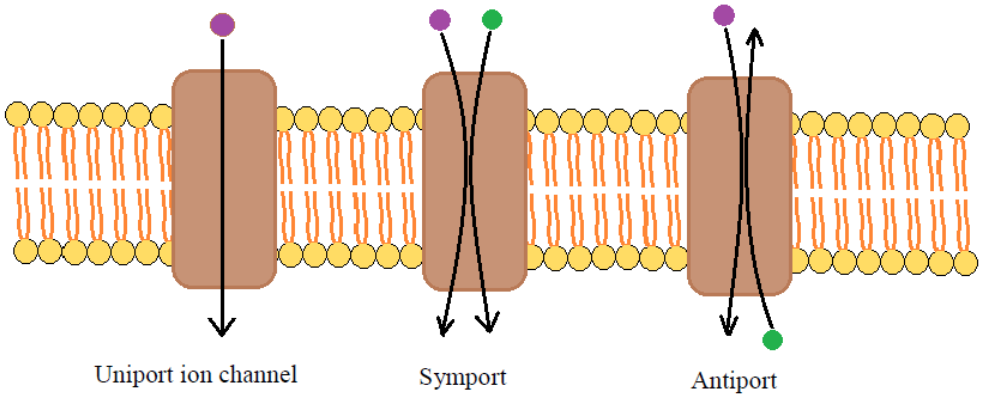

Fig 3.1. Transport of ions across the membrane

3.5 NERNST EQUATION

Nernst equation helps us to understand the concentration effect, electro-potential effect and absorption of ions under the influence of metabolic energy. The electrochemical potential difference across the membrane is designated as Δμ and expressed by the following modified Nernst equation.

$\Delta\mu = \Delta (RT \ln C) + \Delta (zFE)$

Where, z is algebraic valency

Δ (RT lnC) = chemical potential difference due to concentration effect

Δ (zFE) = electropotential difference

R = universal gas constant

T = absolute temperature

C = ion concentration inside and outside the membrane

F = Faraday's constant

E = electropotential in volts on each side of the membrane

The above equation can be simplified as –

$$log \frac{C_i}{C_0} = \frac{-zF\Delta E}{2{\cdot}3RT}$$

Where, Ci = inside ion concentration

C_0 = outside ion concentration

Ci/C_0 is the predicted ratio at equilibrium, when μ = 0, if the actual ratio is greater than the predicted ratio, the cell is performing work to absorb the ions using energy. This is called active absorption. Passive absorption occurs when the actual ratio is equal to or less than the predicted value.

3.6 ANATOMY OF PHLOEM TISSUE

The long-distance movement of dissolved organic substances in angiosperms is accomplished by specialized, elongated cells known as phloem (Figure 3.2). It is composed of four type of cell elements viz. sieve elements, companion cells, phloem parenchyma and phloem fibers. The elements of phloem originate from the procambium of the apical meristem.

A. Sieve elements – It is the most important element of the phloem. In angiosperms, these are called as sieve tubes and in gymnosperms as sieve cells.

Sieve tubes are the long tube-like cells arranged in a row of cells with their end walls perforated in sieve like manner, called as sieve plate. The perforations or sieve areas are also present in their lateral walls. A sieve area in surface view looks like a depression on the wall having a number of dots on the wall and represent enlarged plasmadesmata. Both, sieve plate and sieve areas help to establish the cytoplasmic connections with adjacent cells. Sieve plate areas contain a carbohydrate callose, (β 1-3 glucan) which gets deposited on the wall. Selective autophagy occurs during the maturation process of the sieve tubes resulting in loss of many cell

organelles including the nucleus. The sieve tube elements of many dicot and some monocots contain a special proteinaceous substance of yet unknown function, the P-proteins or Phloem protein bodies.

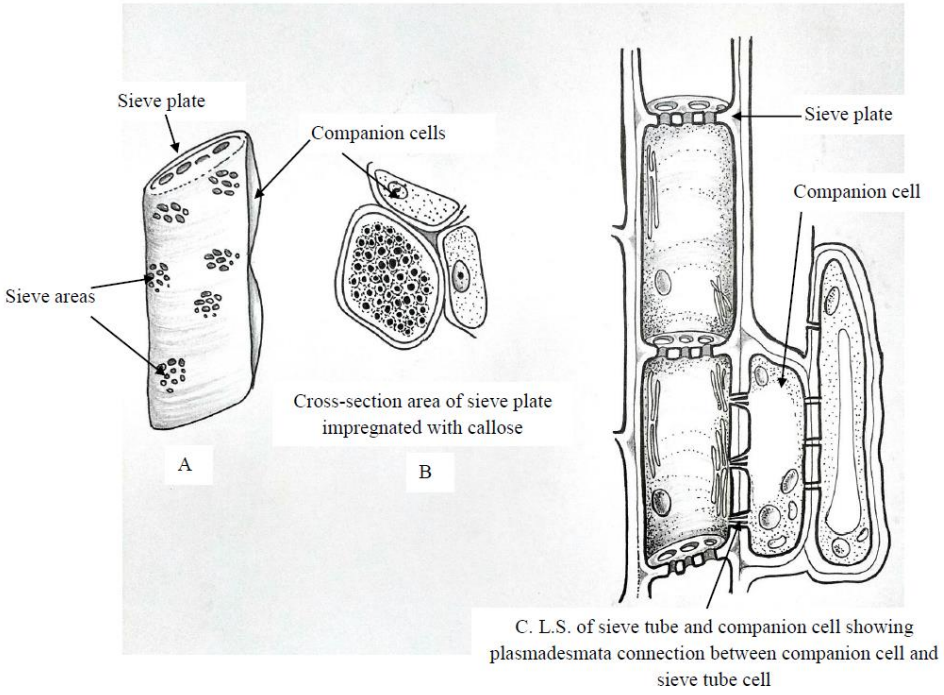

Figure. 3.2 Phloem anatomy; A. A sieve tube with sieve areas and sieve plate, B. Cross section of sieve plate, C. L.S. showing sieve tube and companion cell

A sieve place composed of a single sieve area is called simple sieve place, whereas and end wall containing two or more sieve areas is called as compound sieve plate. Simple sieve plates have transversely oriented end walls, and compound sieve plates have inclined end walls. At maturity there is massive accumulation of the callose over the entire sieve plate areas which render the sieve element non-functional.

Sieve cells are long tubular cells which differ from the sieve tubes in absence of distinct sieve plate on their end wall and lack the P-protein bodies. They are associated with the albuminous cells which are not ontogenetically related to them like companion cells.

B. Companion cells – It is a small parenchymatous cell that is ontogenetically derived from the same mother cell forming the sieve plate by oblique cell division. They are small elongated cells and occur along the lateral wall of the sieve tubes. This cell retains its nucleus and it is supposed that the functions of sieve tube cell are regulated by the nucleus of the companion cell. They communicate through the sieve tubes by means of numerous plasmadesmata pore connections across their common wall.

C. Phloem parenchyma – A good number of parenchyma cells are associated with the sieve elements. These cells are mainly concerned with storage of food material.

D. Phloem fibers – These are long sclerenchymatous fibers found associated with the phloem tissue. They occur in both primary and secondary phloem. These fibers are economically important and are used for manufacturing the ropes and cords.

3.7 MECHANISM OF PHLOEM TRANSPORT

The transport of photosynthates in the phloem is in bidirectional pattern, i.e. form leaves to roots and from leaves to apical meristem (Figure 3.3). The principal photosynthate which is transported through the phloem sap are non-reducing sugars mainly the sucrose sugar. The sugar from leaf mesophyll cells enter into the sieve elements and companion cells of the phloem, this process is called **phloem loading**. The phloem loading occurs through both symplast via plasmadesmata and apoplast. The sucrose uptake via apoplastic way requires the metabolic energy involving sucrose-H^+ symporter. This sucrose and other solutes are translocated away from the source through the sieve elements for long distance transport to the sinks such as developing roots, tubers and reproductive structures. The process by which imported sugars leave the sieve elements of sink tissue is called as **phloem unloading**. Phloem unloading process also involves the transport of sugars via symplastic and apoplastic pathways. The rate of movement of solute transport in phloem is very high from 1to 15 gram per hour per cm^2.The mechanism of phloem translocation in angiosperms can be explained by the Pressure-flow model or Munch hypothesis proposed by the Ernst Munch (1930).

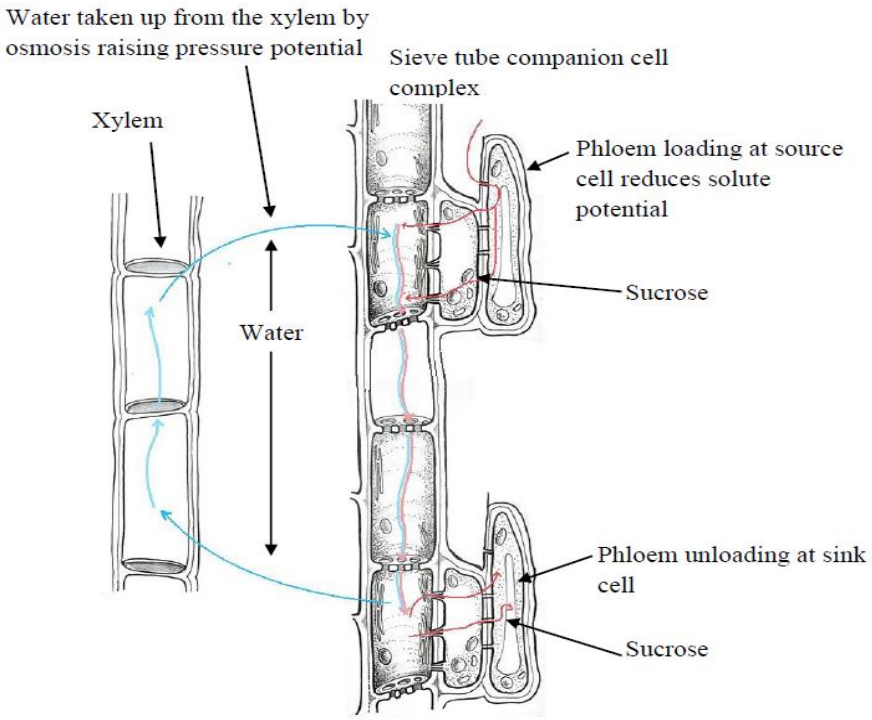

Figure. 3.3. Mechanism of phloem transport

3.8 MUNCH HYPOTHESIS

E. Munch (1930) proposed the pressure-flow hypothesis. It was suggested that the flow of phloem sap in sieve elements is driven by an osmotically generated pressure gradient between source and sink tissue during phloem loading and phloem unloading respectively. The accumulation of sugars at supply end generates a low solute potential (negative) and causes steep decrease in water potential. As a result, water enters into the sieve elements and causes the turgor pressure to increase. At the receiving end, phloem unloading results into lower sugar concentration in the sieve elements generating a higher solute potential (positive) and it results in increase in water potential in phloem cells. As the water potential of the phloem rises above that of the xylem, water tends to leave the phloem in response to the water potential gradient, causing decrease in turgor pressure in sieve elements of the sink tissues. The sieve plates present a continuous series of resistances to the phloem sap and it results into generation of pressure gradient in the sieve elements between the source and sink. The mechanism of phloem transport involves mass flow of the phloem sap which is driven by the pressure gradient. The generation of

pressure gradient is ultimately dependent on the process of phloem loading and unloading (Figure 3.4).

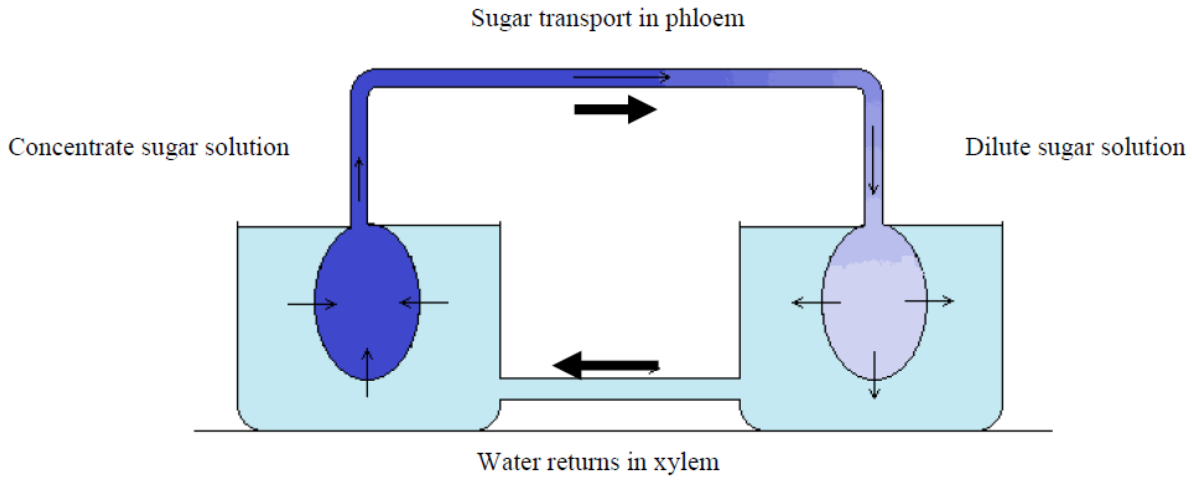

Fig. 3.4 Diagrammatic representation of Munch Hypothesis

3.9 FACTORS AFFECTING TRANSLOCATION

In plants, the translocation through the phloem is affected by several factors discussed below.

A. Temperature – Within physiological limits to the plants, the increase in temperature increases the rate of translocation. However, the optimum range of temperature for translocation is 20-30°C. But above temperature 50°C, translocation decreases with increase in temperature.

B. Effects of Hormones – The phloem translocation is affected by the auxin, Gibberellic acid and cytokinin. They affect the translocation by controlling the growth of sink tissues, leaf senescence and by other developmental processes.

C. Effect of inhibitors – Some metabolic inhibitors like dinitrophenol and cyanides inhibit the translocation in phloem. Effect of inhibitors is known to occur on the transport phenomenon and on the loading and unloading phenomenon.

D. Effect of Potassium and Boron deficiency–Potassium is present in phloem sap in rich amount. According to Spanner (1958) potassium increases the translocation of sugars in sieve tubes by establishing a potential difference across the sieve plates. They are also involved in loading in the minor veins in the leaves. Boron is essential for the sugar transport. It helps in translocation of sugars by forming complex with them, making neutral sugars more electropositive thus making their transport easier through electronegative membranes.

3.10 SUMMARY

In this unit we have discussed thedifferent types of transport of the minerals, the importance mineral transported by the plants, forces involved movement of minerals, the pathway the mineral transport follows, and the mobility of the important minerals.

Let us sum up key points of this chapter –

1. The specific structure and chemical composition of the plasma membrane makes it semi-permeable membrane, allowing it to be selective during the transfer process.

2. The transport across the membrane is either passive without utilization of metabolic energy or active utilizing the metabolic energy of the cell.

3. Simple diffusion is the process of non-mediated transfer of molecules across the membrane along the concentration gradient.

4. Facilitated diffusion involves the help of carrier proteins to cross the membrane by charged molecules

5. Primary active transport utilized the metabolic energy in form of utilization of bond energy of the ATP molecules and transfer the molecules against their concentration gradient.

6.The secondary active transport uses the energy stored in electro-chemical potential gradient in form of H^+ gradient.

7.Nernst equation helps us to understand the concentration effect, electro-potential effect and absorption of ions under the influence of metabolic energy

8. In vascular plants, the phloem helps to transport the nutrients formed during photosynthesis form source (leaves) to sink tissue.

9. In angiosperms, phloem is complex tissue, consisting of sieve tube elements, companion cells, phloem parenchyma and phloem fibers.

10. Sieve tube cells are enucleated living cells having large plasmadesmata in form of sieve areas and sieve plate.

11. The function of sieve tube cells are regulated by the nucleus of the companion cells.

12. In phloem, there is a bidirectional movement of the sap containing sugars.

13. The main sugars in phloem sap are reducing sugar mainly sucrose.

14. The sugars from leaf mesophyll cells enters into the sieve elements and companion cells of the phloem, this process is called **phloem loading**.

15. The process by which imported sugars leave the sieve elements of sink tissue is called as **phloem unloading**

16. The mechanism of phloem translocation in angiosperms can be explained by the Pressure-flow model or Munch hypothesis.

17. The mass flow of the phloem sap which is driven by the pressure gradient and the generation of pressure gradient is ultimately dependent on the process of phloem loading and unloading.

18. Within physiological limits to the plants, the increase in temperature increases the rate of translocation.

19. The phloem translocation is affected by the auxin, Gibberellic acid and cytokinin.

20. Some metabolic inhibitors like dinitrophenol and cyanides inhibit the translocation in phloem.

21. Potassium increases the translocation of sugars in sieve tubes by establishing a potential difference across the sieve plates.

3.11 GLOSSORY

Abscission: The shedding of old leaves through petiole after formation of abscission layer in a living plant**.**

Apoplast: The non-cytoplasmic continuity in a plant consisting of cell wall, inter-cellular spaces and xylem elements

Antiport: Movement of two substances through a transporter protein in relatively opposite direction.

Cambium: A layer of meristematic tissue lying between xylem and phloem responsible for secondary growth in plants.

Diffusion: The tendency of molecules to distribute themselves evenly from higher to lower concentration gradient.

Facilitated diffusion: Relatively faster diffusion process mediated through the transporter protein.

Ion Channels: Transmembrane proteins responsible for transport of particular ion across the membranes cell along the concentration gradient.

Munch hypothesis:E. Munch (1930) proposed the pressure-flow hypothesis to explain the flow of phloem sap in sieve elements.

Phloem loading: The process through which sugars from leaf mesophyll cells enters into the sieve elements and companion cells of the phloem.

Phloem unloading: The process by which imported sugars leave the sieve elements of sink tissue

Plasmodesmata: Channels in plasma membrane connecting adjacent cells through the cell wall and filled with cytoplasm connecting through a tubule called desmotubule.

Primary active transport: The transport of a molecule against its concentration gradient coupled with some exergonic reaction like hydrolysis of ATP into ADP and inorganic phosphate.

Proton Gradient: The electrochemical effect of proton ion gradient across a membrane expressed in terms of electrical potential.

Secondary Active Transport: A transmembrane protein complex that uses energy stored in proton motive force or other ion gradients and operates by symport or antiport.

Symplast: The continuous system of cell protoplast interconnected through plasmodesmata

Symport: Movement of two substances simultaneously in same direction through a transporter protein

Uniport: Transport of only one molecule across the transporter protein across the membrane.

3.12 SELF ASSESSMENT QUESTIONS

3.12.1 Very short answer questions

1. The continuous system of cell protoplast interconnected through plasmodesmata is called?
2. The non-cytoplasmic continuity in a plant consisting of cell wall, inter-cellular spaces and xylem elements is called?
3. The process of non-mediated transfer of molecules across the membrane along the concentration gradient is called?
4. The mineral salts present in the soil solution is absorbed by the plants through?
5. Who proposed the pressure-flow hypothesis to explain the flow of phloem sap in sieve elements?

6. Transport of only one molecule across the transporter protein across the membrane is known as?
7. Channels in plasma membrane connecting adjacent cells through the cell wall and filled with cytoplasm connecting through a tubule are called?
8. Mechanism of active transport can be understood by?
9. Enucleated living cells of the phloem are known as?
10. In gymnosperms, the function of sieve tube is carried out by?

Answer: 1. Symplast, 2. Apoplast, 3., Diffusion 4. Root hairs, 5. Munch, 6. Ion channels, 7. Plasmodesmata, 8. Nernst equation, 9. Sieve tube, 10. Sieve cells

3.12.2 Multiple choice questions –

1. The absorption of salts by the roots is by
a) Symplast b) Apoplast
c) Both a and b d) None of these

2. The mineral movement through apoplast pathway is based on
a) Diffusion b) Osmosis
c) Ion channels d) All of the above

3. The mineral movement through symplastic pathway is
a) Active b) Passive
c) Direct d) Through the xylem

4. The symplastic movement of the minerals from one cell to another takes place through
a) Cell wall b) Vacuole
c) Intercellular spaces d) Plasmadesmata

5. Apoplast of the plant includes –
a) Cell wall b) Intercellular spaces
c) Xylem elements d) All of the above

6. The force responsible for upward movement of minerals inside the xylem elements is
a) Hydrostatic pressure b) Osmotic pressure
c) Electrostatic pressure d) Transpirational pull

7. ATP hydrolysis during transportation process take place during –
a) Diffusion b) Facilitated diffusion
c) Active transportation process d) All of the above

8. Which character of the transporter protein is correct

a) They are multimeric proteins b) They span the plasma membrane several times
c) Form transient bond with their substrate d) All of these

9. Transport of substances against the concentration gradient can takes place by
a) Facilitated diffusion b) Primary active transport
c) Secondary active transport d) Both b and c

10. The energy stored in electro-chemical potential gradient to transport the substances is used for
a) Primary active transport b) Secondary active transport
c) Diffusion d) None of the above

11. The concentration effect, electro-potential effect and absorption of ions under the influence of metabolic energy can be understood by
a) Munch hypothesis b) Pressure flow hypothesis
c) Nernst equation d) None of the above

12. The function of sieve tube is regulated by
a) Sieve cells b) Companion cells
c) Albumen cell d) Phloem parenchyma cells

13. P-proteins are found associated with
a) Sieve tube b) Sieve cells
c) Albumen cells d) Phloem fibers

14. The phloem unloading process involves
a) Oscillation of sieve tubes of leaves b) Symplastic movement of ions
c) Apoplastic movement of ions d) Both b and c

15. The mechanism of phloem transport involves-
a) Mass flow of phloem sap b) Generation of pressure gradient
c) Process of phloem loading and unloading d) All of the above

Answer Key :1. c, 2. a, 3. a, 4. d, 5. d, 6. d, 7. c, 8. d, 9. d, 10. b.11. c, 12. d, 13. a, 14. d, 15. d.

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3.15 TERMINAL QUESTIONS

3.14.1 Long answer type questions

1. Describe the methods of passive transport in the plant cells.
2. What are ion channels? Describe the importance of ion channels for transport of minerals in plants?
3. Describe the different components of the phloem tissue?
4. What do you mean by phloem loading and unloading? Describe its importance for the plants.
5. How the Munch hypothesis explains the mechanism of transportation process in phloem tissue?

6. How the symplast and apoplast continuum of the plant cells help in transport of the minerals in plant? Discuss in detail.
7. What are the different methods for absorption of minerals against the concentration gradient in plants?

3.14.2 Short answer type questions

1. Describe the difference between simple diffusion and facilitated diffusion?
2. Write short note on ion channels.
3. What are symport and antiport transport?
4. Mention the difference between the symplast and apoplast.
5. Give a brief account Nernst equation and its importance.
6. What is difference between sieve tube sieve cell?
7. What are the factors affecting translocation in the plants?
8. Write a short note on pressure flow hypothesis.

BLOCK-2

PLANT METABOLISM

UNIT-4-PHOTOSYNTHESIS AND PHOTORESPIRATION

Contents:

4.1 OBJECTIVES

To study about the process of photosynthesis in green plants in respect of photosynthetic pigments to absorb light at different spectrum to absorb energy and transmit it to neighboring accessory molecules. This chapter also focused on electron transport and the process of photophosphorylation resulting in ATP synthesis. To learn about the carbondioxide fixation among C_3 and C_4 plants and understand the importance of photorespiration in plants.

4.2 INTRODUCTION

Terrestrial plants as well as marine cyanobacteria are the familiar photosynthetic organisms which carry out photosynthesis, an energy generating electrochemical process needed for various chemical reactions through the transfer of electrons and protons among different carriers and substrates within the plants. The process starts with insubstantial things like light and air generating nourishment providing products with a major byproduct, oxygen. Photosynthesis is the process of generating fuel within the green plants by utilizing photons as energy carriers from the light energy and carbon dioxide from air. The fuel is generated in the form of organic compounds. The plants which perform photosynthesis are called autotrophs and they are the producers of ecosystems. Another group of organisms which feed on both other plants and animals are referred to as heterotrophs. All the other beings in the ecosystem harness energy and obtain food by feeding on them and are called herbivores. Organisms who feed on herbivores are called carnivores and all these organisms belonging to different habits of obtaining food contribute to the food web or food chain of the ecosystem.

4.3 PHOTOSYNTHESIS

Photosynthesis is a usual process in which light energy is used to reduce carbon producing carbohydrates which in turn serve as energy fuel directly for the photosynthetic organisms which produced them and indirectly for the non-photosynthetic organisms. The process of photosynthesis involves the conversion of light energy into chemical energy. In the twentieth century, it was believed that the reduction of CO_2 was directly performed through the photosynthetic pigments which on reacting with water to form carbohydrate but now it is known that photosynthesis is a two-stage process:

Stage 1 involves splitting of water molecules into molecular oxygen and protons.

$$2\,H_2O \xrightarrow{\text{light}} O_2 + 4\,[H\bullet]$$

Stage 2 involves condensation of carbon dioxide with the protons released from the water splitting with the yield of organic compounds and a molecule of water.

$$4\,[H\bullet] + CO_2 \longrightarrow (CH_2O) + H_2O$$

The overall two staged of photosynthesis can be summarized by the reaction below:

$$CO_2 + H_2O \xrightarrow{\text{light}} (CH_2O) + O_2$$

The two stages of photosynthesis are divided into light stage reaction and dark stage reaction. The light dependent reaction or light reaction used the direct sunlight to carry out the reaction in light whereas the dark reaction occurs in dark but the enzymes which played vital role in this process are indirectly activated due to the light and hence the term dark reaction is considered to be a misnomer. The light reaction occurs in grana and dark reaction occurs in the stroma of chloroplast. In the light dependent reaction, the light energy excites electrons from ground energy state to high energy state to form ATP and NADP. In the process, water is used to split into molecular oxygen as a by-product of the reaction. The energy molecules, ATP and NADP are used to form carbon-carbon (C-C) bonds in the carbohydrates. In the light independent reaction, carbon dioxide is fixed into carbohydrate as a process of carbon fixation in the environment by ribulose bisphosphate (RuBP) which is a 5-C chemical compound. The conversion of carbon dioxide into carbohydrates is not simple and living cells cannot convert and utilize light energy directly. For this process to happen, a series of complicated reactions form a cascade to convert CO_2 into C-C bond energy. Light independent or dark reactions are also called carbon-fixing reactions. In the primitive structure of autotrophs belonging to the single celled stage, carbon dioxide enters through simple diffusion but plants with complex cell arrangement have specialized structures like guard cells and stomata for air and gas diffusion. The calvin cycle occurs in the stroma of chloroplasts.

4.4 HISTORY

The process of photosynthesis has existed from the beginning of time but it was unknown to human knowledge until the 1800s. In the 1600s, Jan Baptista van Helmont, a Belgian chemist, physiologist and physician performed a five-year experiment on a willow tree by planting it in a pot and providing water for five years in a controlled environment. Helmont concluded that it was water and not the soil which was responsible for the growth of the tree by providing necessary nutrients to it. This study was not entirely correct but it led to the partial discovery of photosynthesis and the importance of water in it. The study of evolution of larger animals debates the involvement of atmospheric oxygen attributed to the photosynthetic activity of cyanobacteria although production of oxygen through plants had been a matter of debate until 1771 when Joseph Priestly proved it through a simple experiment. He observed the candle in the jar was burned out after some time and this could be prevented by introducing a small plant into the jar. To understand more, Priestly performed a series of experiments which led to the discovery of oxygen. Priestly placed a burning candle and a mouse within a bell jar and observed that after some time of candle burning out, mouse dies due to suffocation. Next, Priestly

introduced a small mint plant with a mouse and a burning candle into the bell jar. The mouse continued to live as air was never depleted by burning candle and kept on restoring through the live plant whereas the candle continued to burn. Priestly concluded that burning candle and air was altering the atmospheric air within the bell jar and the green plant was somehow presenting the change and restoring the air. In 1774, Priestley's experiment results were published in "Experiments and Observations of Different Kinds of Air, Volume I" which later led to the discovery of oxygen in the air.

Another study by Jan Ingenhousz, a Dutch chemist, biologist and physiologist in the late 1770s revealed that plants produce oxygen. The submerged plant was allowed to grow under sunlight and shade and the comparative study revealed that plants in sunlight produce bubbles but it was absent when the same plant was transferred to shade. It revealed that plants produce oxygen under the presence of sunlight through the process of photosynthesis. Other series of experiments via different scientists helped in supporting the above discoveries. In 1796, Jean Senebier, a Swiss botanist, pastor and naturalist demonstrated that plants absorb carbon dioxide and release oxygen. It leads to the discovery involving the importance of carbon dioxide in plants. In the early 1800s, Nicolas-Théodore de Saussure informed that plants growth and increased mass of growing plants is resulting due to uptake of water and not from the carbon dioxide. In the 1840s, Julius Robert Mayer, a German physician and physicist's discovery revealed the first law of thermodynamics which states that energy can be neither created nor destroyed which further divulges in proposing that plants convert light energy into chemical energy. Julius Sachs in 1862-64 investigated that starch is produced under the presence of sunlight in relation to chlorophyll. A general equation of photosynthesis was proposed by Cornelis Van Niel in 1930s which is given by:

$$CO_2 + 2\ H_2A + \text{Light energy} \longrightarrow [CH_2O] + 2\ A + H_2O$$

This reaction was modified later which transformed it into much simplified and known reaction:

$$6\ CO_2 + 6\ H_2O + \text{Solar energy} \longrightarrow C_6H_{12}O_6 + O_2$$

From the experiment performed in 1932 by Robert Emerson and William Arnold on Chlorella cells, it was revealed that the excitement of 2500 chlorophyll molecules led to the production of single O_2 molecules. In 1957, Robert Emerson introduced an eponymous effect called Emerson effect in which when plants are exposed to wavelength greater than 680 nm, only one photosystem is activated at PS700 which results in the formation of ATP only. It was also observed that at a wavelength less than 680 nm, the rate of photosynthesis decreases. Emerson further concludes that providing both shorter and longer wavelength altogether enhances the rate of photosynthesis resulting in higher yield in which both photosystem I and II operate simultaneously. In 1954 Daniel Arnon and his colleagues enlightened the concept of ATP production from ADP and P_i during photosynthetic electron transfer in spinach chloroplasts. This study was supported by Albert Frankel through the discovery of light-dependent ATP production

in membranous structures called chromatophores derived from photosynthetic bacteria. The study revealed that light energy is captured in these photosystems of these photosynthetic organisms and is transformed into phosphate bond energy of ATP through the process of photophosphorylation.

4.5 PHOTOSYNTHETIC PIGMENTS

The light energy is absorbed by the reaction centre to trap the energy which excites electrons from ground state to excited state and transferred to other acceptor molecules. Different photosynthetic pigments absorb light at specific wavelengths. Major photosynthetic pigment, chlorophyll a is responsible for major absorbance but it also works on specific range of wavelengths and light energy on other wavelengths is simply lost. Also, within the spectrum range of 450 and 650 nm, many photons escaped from absorbing light energy suggesting the low density of chlorophyll a. Accessory pigments served the purpose of effective absorbance at different wavelengths than from the visible range. Accessory photosynthetic pigments include Chlorophyll b and carotenoids as vital light-harvesting molecules which transfer energy to the reaction centers. Chlorophyll b contains a formyl group in the place of methyl group in molecular structure of chlorophyll a. The small difference causes the change in the absorbance peaks directing it towards the centre of the visible region. Chlorophyll b absorbs light at wavelength between 450 and 500 nm whereas carotenoids show absorbance between 400 and 500 nm. The yellow and red color of flowers and fruit is caused due to the presence of carotenoids which results in brilliant shades and color within them. Other than transferring energy to reaction centers, Carotenoids perform other functions of suppressing photochemical reactions especially the one involving the oxygen through the induction of bright sunlight. Carotenoids prevent plants from light and oxygen exposure and the plants which lack carotenoids are threatened and quickly killed. Light-harvesting complexes are formed by various accessory pigments surrounding the reaction centers. The most abundant protein is the 26-kd subunit of light-harvesting complex II (LHC-II) in chloroplasts. Seven chlorophyll a molecules, six chlorophyll b molecules and two carotenoid molecules bind to this subunit.

In sea and oceans, little blue and red light is absorbed by the water and the chlorophyll molecules residing on the surface and it reach the algae at a depth of meter or more in the ocean. Phycobilisomes are large protein assemblies present in Cyanobacteria (blue-green algae) and red algae harvest green and yellow light that infiltrate to their ecological niche. Phycobilisomes are present on the outer surface of the thylakoid membrane and act as light-absorbing antenna which helps in channel excitation energy into the reaction center of photosystem II. The light energy is absorbed at 470 to 650 nm wavelength and this range lies in between the blue and far-red absorption peaks of chlorophyll a. Many phycobiliprotein subunits comprise the large assemblies of phycobilisomes containing many prosthetic groups attached to each one covalently called bilin and linker polypeptides. Hundreds of bilins are present in phycobilisomes and phycocyanobilin and phycoerythrobilin are the most common examples. The phycobilisomes are designed in such

a way so that light energy could be trapped in algae to occupy ecological niches when other organisms depend only on chlorophyll for trapping the light energy.

4.6 NATURE OF LIGHT

Sunlight is composed of white light which separates into different colors by passing it through the prism. Different colors correspond to different wavelengths belonging to different spectrums of light. The light rays travel in wave form making troughs during travel and the distance between two travels is defined by wavelength. Lights of different spectrum have different wavelengths and so does different energy (Figure 4.1). Energy is inversely proportional to the wavelength which implies that longer the wavelengths have less energy whereas shorter wavelengths have more energy. Different colors in the spectrum of light determine the different wavelengths and the visible light falls under the electromagnetic spectrum. The longer wavelength of the visible light corresponds to the red spectrum and shorter wavelength of the visible light corresponds to the violet spectrum. The wavelength of light longer than the red spectrum of the visible light is called infrared; the wavelength of light shorter than the violet spectrum of the visible light is called ultraviolet. As mentioned earlier light travels in the form of waves and hence its nature is similar to that of waves and as well that of particles. Due to the wave nature of light, the light rays have tendency to bend when the density of the environment they are passing through changes and they bend themselves accordingly to or away from the theoretical perpendicular axis. The particle nature depicts that light rays are made up of particles which could be charged and carry photons and electrons in them. This causes a photoelectric effect when zinc is exposed to ultraviolet rays; it becomes positively charged as light rays are responsible for forcing electrons out from the zinc. The wavelength of light at which photoelectric effect is caused is called the critical wavelength.

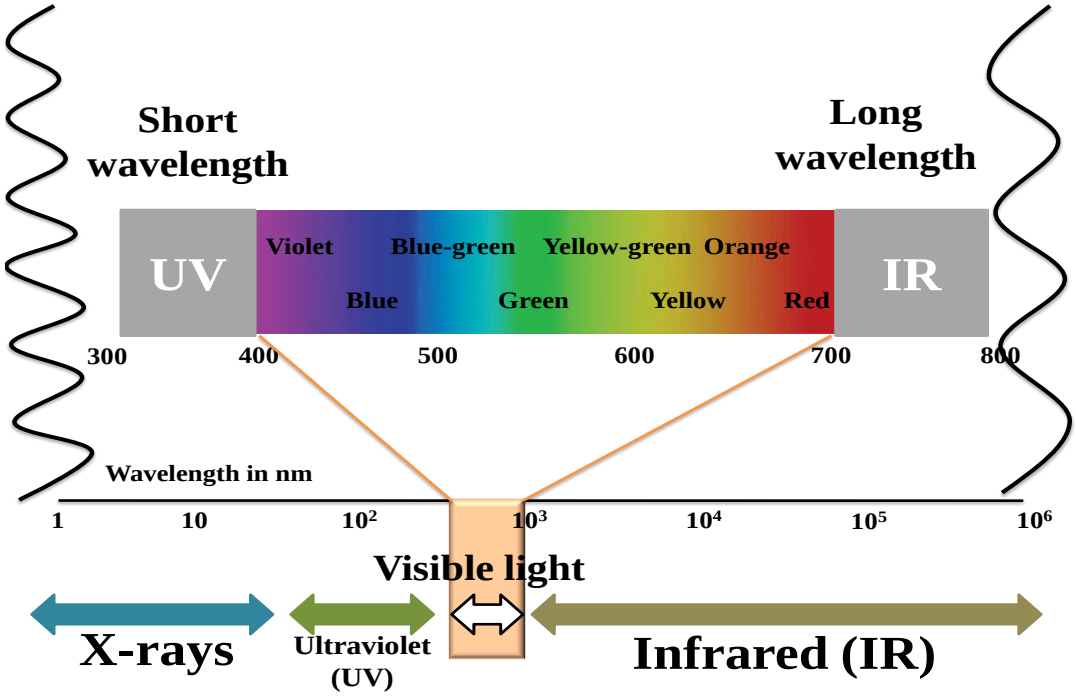

Figure 4.1: The spectrum of electromagnetic radiation.

4.7 EMERSON EFFECT

Emerson and his coworkers studied different light spectra in order to obtain the best spectrum to increase the rate of photosynthesis. The study revealed that the combination of two combined beams produces more oxygen than the average of the sum of individual beams. This enhancement in the improved photosynthetic efficiency of far-red (700 nm) by simultaneous illumination with orange-red light (650 nm) is referred to as the Emerson effect. The Emerson effect (*E*) can be expressed as the ratio of the rate of oxygen evolution in the far-red light in the presence of a supplementary beam and the same rate in the absence of it.

$E = \Delta O_2$ (in combined beams) - ΔO_2 (short-wave beam alone) / ΔO_2 (long-wave beam alone)

In order to study different action spectrums, the graphical representation can be helped to compare different spectrums individually and in combination through the plots of *E* versus the wavelength of supplementary light.

4.8 TWO PIGMENT SYSTEMS

The light energy absorption in terms of photons creates an energy difference when electrons are excited from ground state to excited state and this energy at excited state is called excitation energy which can be transferred from one molecule to another neighboring molecule. The

process of transferring excitation energy from one molecule to another is termed as resonance energy transfer and it depends on the distance between the donor and acceptor molecule. In order to conserve energy, the donor must be in an excited state while transferring energy to an acceptor of equal or lower energy. It is a fact that the special pair of chlorophyll molecules is in lower energy at excited state than that of single chlorophyll molecules by which energy is trapped to reaction centers from other molecules. The light-absorbing pigments of thylakoid or bacterial membranes are placed in efficient arrangement for the electrons to excite and then transferred towards an approved cascade of molecules and this array is called photosystem; is of two kinds naming, photosystem I and photosystem II. Different pigment molecules are arranged in a photosystem which are designed to absorb photons. Few pigment molecules are associated with the photochemical reaction centre from which electrons are excited and remaining are arranged in light-harvesting or antenna molecules through which energy is transmitted in a photosystem. Thylakoid membranes are differentiated into two kinds of compartments; stacked and unstacked regions. Due to the stacking inside the thylakoid, the overall volume increases in chloroplast. Photosynthetic assembly differs in stacked and unstacked regions within the thylakoid. Stacked region abode Photosystem II whereas Photosystem I and ATP synthase lie in an unstacked region although a cytochrome bf complex is found in both stacked and unstacked regions. The electron transport and energy transfer travel back and forth within the stacked and unstacked region, in which plastoquinone and plastocyanin are the carriers of electrons within the different regions of thylakoid. The protons released by photosystem II in stacked regions are accumulated within the common internal thylakoid space to be utilized by ATP synthase located at the unstacked region of thylakoid.

4.9 PHOTOSYNTHETIC UNIT

Various chlorophyll pigment molecules and other accessory pigment molecules are arranged in array to form a photosystem which is composed of a number of accessory molecules, which absorb photon and light energy to pass it on to other molecules (Figure 4.2). The pigment molecules like chlorophyll have light absorbing properties which are quickly released as fluorescence and heat when experimented in vitro but in vivo, very little fluorescence from the release of energy is observed. It is due to the fact that most of that energy is passed on to the neighboring chlorophyll molecule through the excitation from the ground state to the excited state in the molecule which has received energy and the molecule which has passed on the energy returns to the ground state once again. In this way, the energy is continued to pass on through the various antenna molecules through the excitation and then later return to the ground state until it reached a special molecule pair of chlorophyll at the photochemical reaction center which causes its excitation so that an electron is promoted to the higher energy orbital. From the reaction centre, this excited electron passes to the nearby electron acceptor leaving the reaction-center chlorophyll empty giving rise to electron hole due to missing electron and it acquires a positive charge and on the other hand the molecule which has accepted the excited electron

becomes electron rich acquiring a negative charge. In the reaction centre, the loss of electrons is compensated quickly by the neighboring electron donor molecule and this becomes the basis of initiation of redox or oxidation-reduction chain.

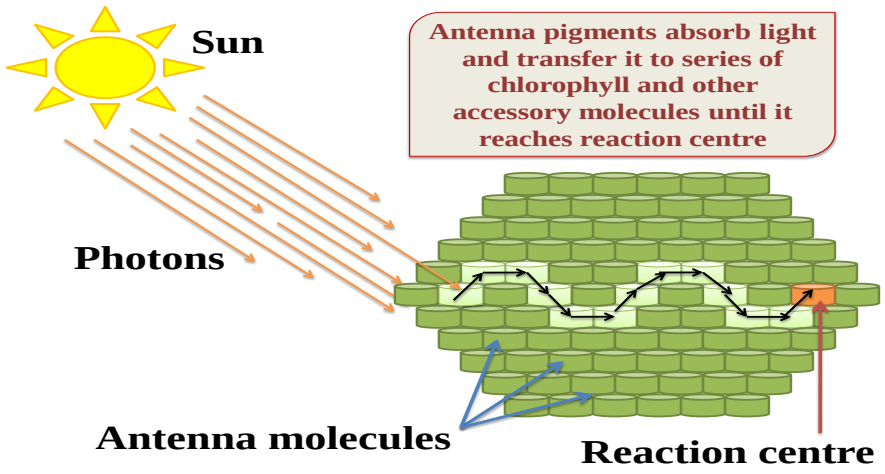

Figure4. 2: The diagrammatic representation of photosynthetic units.

4.10 Z-SCHEME

There are two kinds of photosystems with their own reaction centers and accessory molecules but they work in tandem fashion and some of their electron acceptors are common which cause the electron transfer to become a cyclic process. Photosystem II (PS II) is a pheophytin-quinone type of system with almost equal amounts of chlorophyll a and chlorophyll b molecules. Its reaction centre P680 absorb photons at 680 nm wavelength to excite at excited state, P680* and drives electrons through the cytochrome b_6f complex with an affiliated faction of moving protons across the thylakoid membrane. In Photosystem I (PS I), the reaction centre P700 transfers electrons on absorbing photons from sunlight to the Fe-S protein ferredoxin and then later to $NADP^+$ which results in the yield of NADPH. The Z-scheme explains the pathway of electrons flow from H_2O to $NADP^+$ giving rise to the non-cyclic pathway of photosynthesis (Figure 4.3). The Z-scheme can be summarized through the equation as below:

$$2\ H_2O + 2\ NADP^+ + 8\ photons \longrightarrow O_2 + 2\ NADPH + 2\ H^+$$

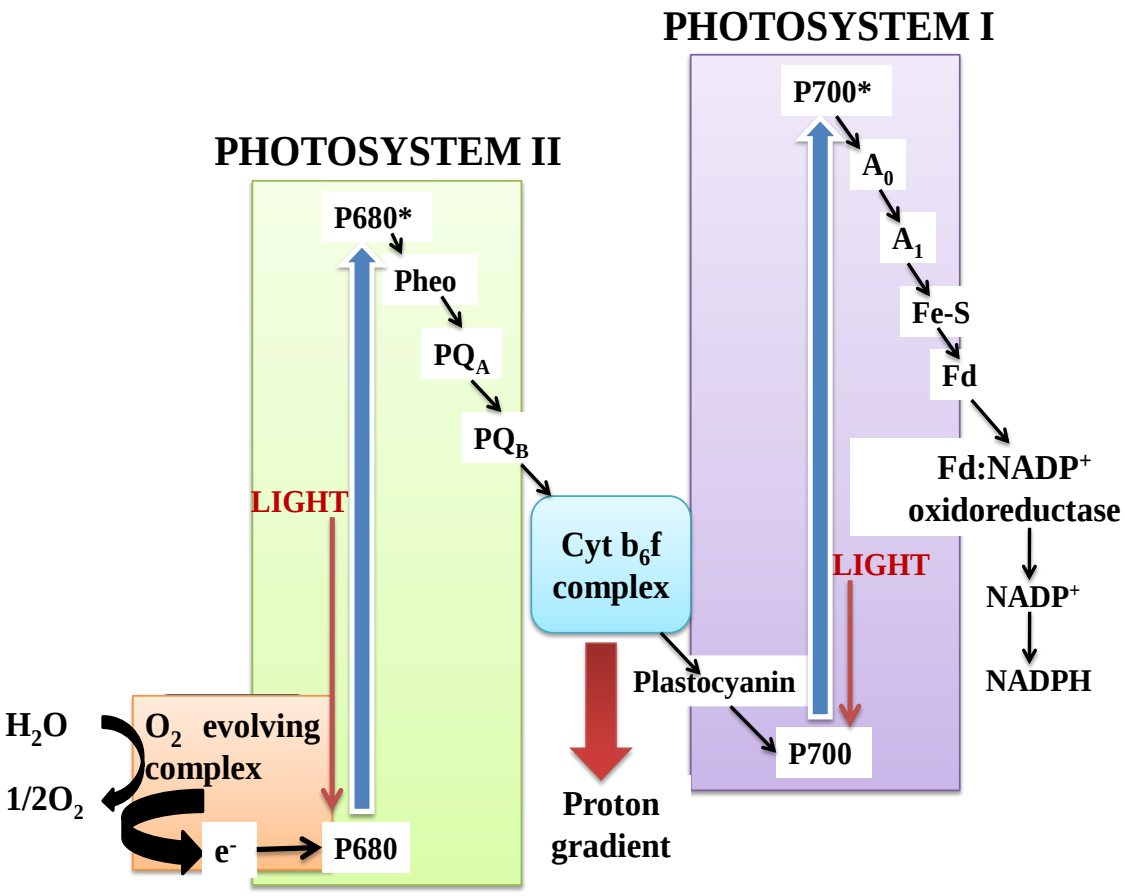

Figure 4.3: Photosystem I and Photosystem II in the Z-scheme of Chloroplast.

4.11 ELECTRON TRANSPORT AND PHOTO PHOSPHORYLATION

In the Z-scheme of electrons transport, electrons move from water to $NADP^+$ in plant chloroplasts, 12 H^+ moves from stroma to the thylakoid membrane at the expense of four electrons and per O_2 formation. Eight protons are allowed in the thylakoid membrane through cytochrome b_6f complex and four electrons are passed through an oxygen-evolving process. A proton gradient is formed inside the lumen (P side) of chloroplast. Minimum eight electrons are needed to be transported from H_2O to NADPH that means one photon per electron at each reaction center. This process is called non-cyclic photophosphorylation (Figure 3) and can be summarized as below:

$$2\ H_2O + 8\ photons + 2\ NADP^+ + \sim 3\ ADP + \sim 3\ P_i \longrightarrow O_2 + \sim 3\ ADP + 2\ NADPH$$

There is another way of electron flow which is cyclic in nature and in which the ratio of NADPH to ATP formed in the light is varied and the process is called cyclic electron flow. Where non-

cyclic electron flow involves both PS I and PS II and electrons flow through tracing the path of Z-scheme, cyclic electron flow involves only PS I. The reaction center, P700 absorb light energy in the form of photons and the electrons are passed the reaction center to ferredoxin but do not trace the path towards $NADP^+$ but instead return the PS I through the cytochrome b_6f complex and then to plastocyanin. Plastocyanin then acts as electron donor and donates electrons to P700 and then later transferred to ferredoxin molecule and in this manner, the cyclic electron flow through PS I continues. The cyclic electron flow does not involve the formation of NADPH and the evolution of O_2 but the process in turn engages in proton pumping by the cytochrome b_6f complex and by phosphorylation of ADP to ATP. This process is called cyclic photophosphorylation (Figure 4) and equation below summarizes the process:

$$ADP + P_i \longrightarrow ATP + H_2O$$

The electrochemical gradient formed inside the lumen (P side) of the chloroplast is responsible for generating ATP through enzyme complex ATP synthase. The ATP synthase is a large complex made up of two functional components, CF_0 and CF_1. CF_0 is a transmembrane and CF_1 is a peripheral membrane protein of the chloroplast. The structure looks like a knoblike projection from the outside stroma (N side) of the thylakoid membrane. F_1 portion of ATP synthase is alkaline from which protons flow down the concentration gradient from lumen to stroma region of chloroplast across the thylakoid membrane from P to N side of chloroplast. This proton flow drives a force which is responsible for condensing ADP and P_i and on their binding; ATP is released through ATP synthase.

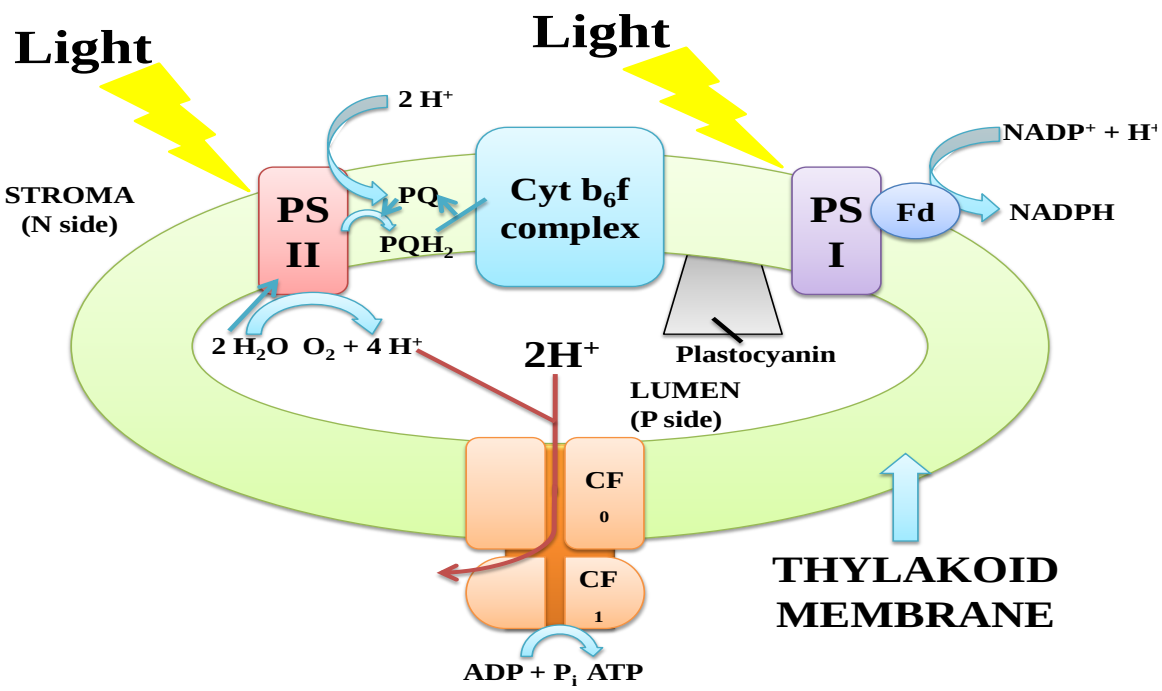

Figure 4.4: Electron and proton flow through photosystem and cytochrome b_6f complex and production of electrochemical gradient across the thylakoid membrane.

4.12 CARBONDIOXIDE FIXATION AND REDUCTION- CALVIN BENSON PATHWAY

The photosynthetic green plants use enzymatic machinery to catalyze the conversion of CO_2 into simple organic compounds through a process of CO_2 assimilation or CO_2 fixation or carbon fixation. Carbon dioxide is assimilated through a cyclic pathway elucidated in 1950s by Melvin Calvin, Andrew Benson and James A. Bassham and which is more popularly known as the Calvin cycle or the photosynthetic carbon reduction cycle. The CO_2 assimilation occurs in plastids which are self-replicating organelles bounded by double membrane and containing some important genes which encodes some proteins needed by the organelle during its genomic processing. Carbon dioxide assimilation occurs in three steps (Figure 4.5). The first stage is named fixation in which CO_2 is condensed using ribulose 1, 5-bisphosphate to produce two molecules of 3-phosphoglycerate. In the next step of reduction, the result of the first step, 3-phosphoglycerate is reduced to triose phosphates (glyceraldehydes-3-phosphate and dihydroxyacetone phosphate). In this manner three molecules of CO_2 are fixed with three molecules of ribulose 1, 5-bisphosphate to generate six molecules of glyceraldehydes-3-phosphate in equilibrium with dihydroxyacetone phosphate. In the third step, regeneration of the acceptor, regeneration of ribulose 1, 5-bisphosphate is achieved by five out of the six molecules of triose phosphates. The sixth molecule of triose phosphate is used in production of hexoses to

be used in various energy generating metabolic processes such as glycolysis, starch or sugar synthesis.

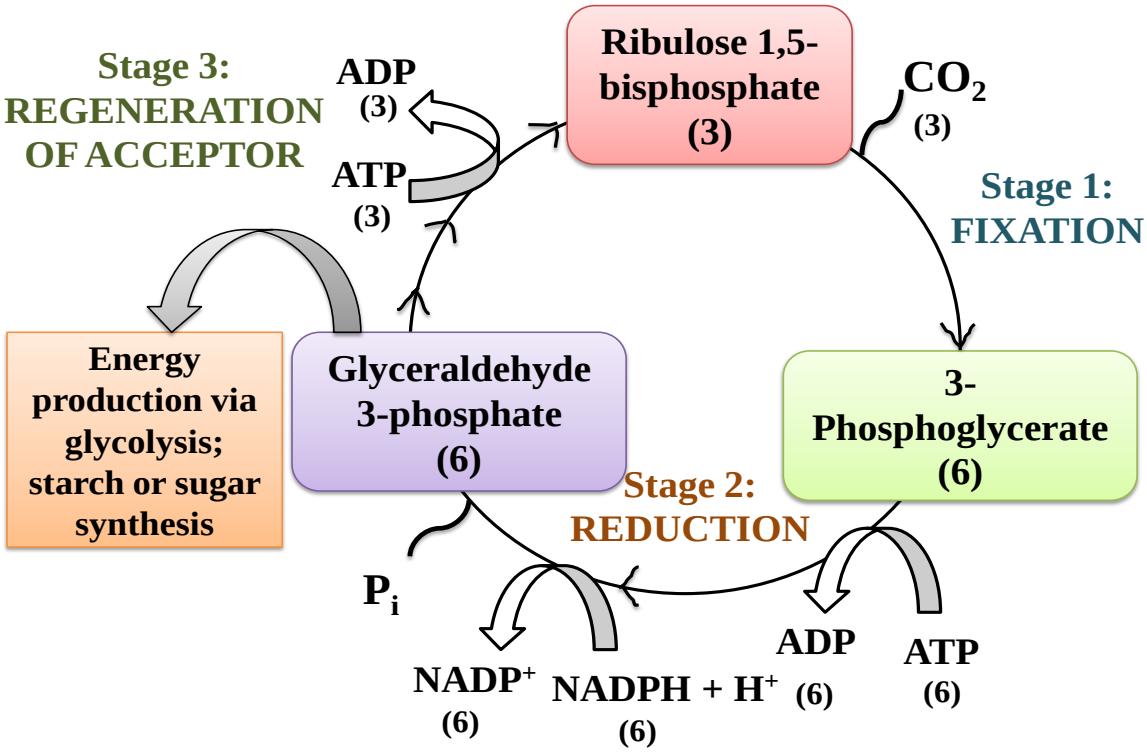

Figure 4.5: Different steps involved in the Calvin cycle.

The entire process begins with the fixation of CO_2 into 3-phosphoglycerate. The production of these three carbon compounds is responsible for naming the plants involved in this process as C_3 plants. Ribulose 1, 5-bisphosphate carboxylase/oxygenase (Rubisco) is the enzyme which is responsible for catalyzing the CO_2 incorporation into organic form. It is the carboxylase activity of rubisco which covalently attaches CO_2 to the five carbon sugar ribose 1, 5-bisphosphate to form an unstable six carbon intermediate compound which is cleaved to form two molecules of 3-phosphoglycerate for each one molecule of CO_2 and one molecule of ribulose 1, 5-bisphosphate. Rubisco has a complex structure made up of eight identical subunits with molecular weight of 53,000, a catalytic site and eight identical small subunits of molecular weight of 14,000. The enzyme has an unexpectedly low turnover number; when three molecules of CO_2 are fixed per second per molecule of enzyme at 25^oC. Hence, Rubisco is present in great abundance in the biosphere and 50% of soluble proteins in chloroplasts are involved in making rubisco enzymes. Rubisco is made up of carbamylated Lys side chain with a central bond ion, Mg^{2+} (Figure 6). The Mg^{2+} is present in the active site of enzymes and catalyzes the reaction by polarizing CO_2. The active site opens up for nucleophilic attack by the five-carbon enediolate

reaction intermediate formed on an enzyme which later breaks down due to instability into two molecules of 3-phosphoglycerate. Rubisco is the prime target for regulating this pathway and it remains inactive until carbamylated by binding tightly to the active site and locking the enzyme in closed conformation, in which Lys^{201} is inaccessible. It is the activity of Rubisco activase which promotes ATP-dependent release of the ribulose 1,5-bisphosphate which leads in exposing the Lys amino group to non-enzymatic carbomylation by CO_2. Now Mg^{2+} is available for binding and rubisco is hence activated. Also, a naturally occurring transition state analog during dark reaction, 2-carboxyarabinitol, is considered as a nocturnal inhibitor with structure similar to β-keto acid, and acts as potent regulatory molecule for rubisco activity. The analog is formed as an intermediate of the rubisco reaction and has potential in inhibiting carbamylation rubisco. It is formed during dark reaction and when exposed to light, it is either disintegrated to become inactive or is barred by rubisco activase to activate rubisco.

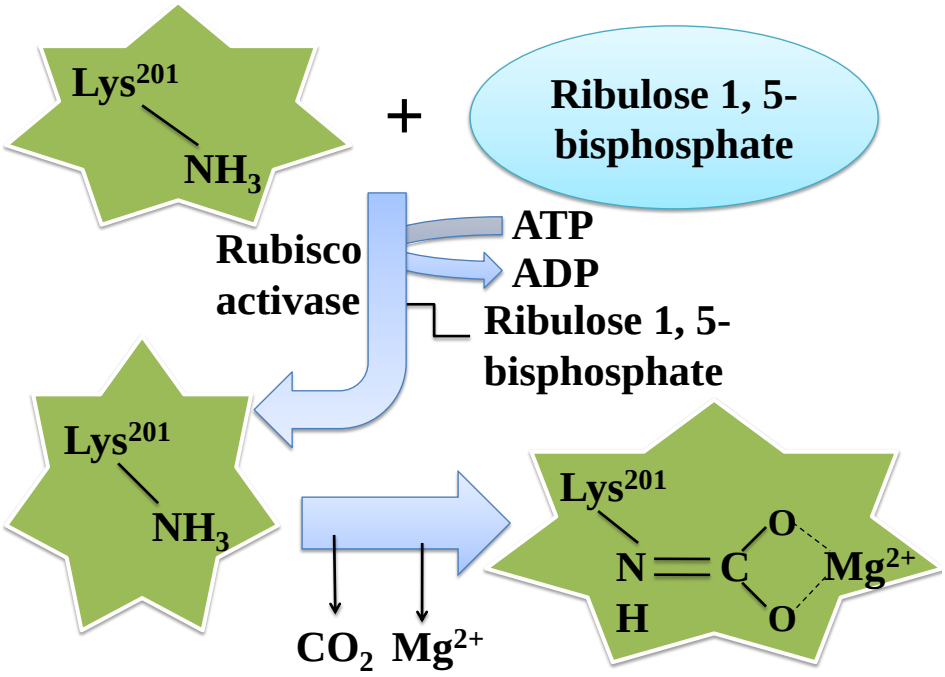

Figure 4.6: The carboxylase activity of enzyme Rubisco.

Two steps are involved in converting 3-phosphoglycerate to glyceraldehyde-3-phosphate. In the first step of stage 2 process, transfer of phosphoryl group is catalyzed by stromal enzyme, 3-phosphoglycerate kinase from ATP to 3-phosphoglycerate. Chloroplast specific isozyme, glyceraldehydes-3-phosphate dehydrogenase catalyzes the donation of electrons from NADPH which lead to the production of glyceraldehydes-3-phosphate and P_i. Another enzyme called Triose phosphate isomerase catalyzes the interconversion of glyceraldehydes-3-phosphate and dihydroxyacetone phosphate in equilibrium. The six molecules of triose phosphates are produced in each cycle and most of the molecules are involved in regenerating the ribulose 1, 5-

bisphosphate and rest is involved in starch synthesis or for storage for later use. Triose phosphates can also be involved in various biochemical metabolic processes like glycolysis. The regeneration of ribulose-5-phosphate is important for the cyclic process to continue. There are various intermediates including three-, four-, five-, six- and seven- carbon compounds (sugars). It starts with enzyme transaldolase which catalyzes the reversible condensation of glyceraldehydes-3-phosphate with that of dihydroxyacetone phosphate to generate fructose 1, 6-bisphosphate. Enzyme fructose 1, 6-bisphosphatase (FBPase-1) cleaves fructose 1, 6-phosphate into fructose-6-phosphate releasing an inorganic phosphate as a result, making it as a first irreversible step of the third stage process of CO_2 assimilation. At next step, transketolase catalyzes the transfer of two carbon group from a ketose sugar acting as a donor, fructose-6-phosphate to an aldolase sugar acting as an acceptor, glyceraldehyde-3-phosphate producing pentose sugar xylulose-5-phosphate and a tetrose sugar, erythrose-4-phosphate. Enzyme transketolase contains thiamine pyrophosphate (TPP) acting as a prosthetic group and it needs Mg^{2+} for its activity. TPP acts as a temporary carrier for the two-carbon fragment from a sugar compound for transferring it to another sugar as an electron sink for the reaction to take place. Enzyme transaldolase again condenses two sugars and upon the combination of dihydroxyacetone phosphate, a triose sugar phosphate and erythrose-4-phosphate, a tetrose sugar phosphate, a seven-carbon sugar called sedoheptulose 1, 7-bisphosphate is produced. Enzyme sedoheptulose 1, 7-bisphosphatase which is a unique enzyme to plastids cleave substrate sedoheptulose 1, 7-bisphosphate into sedoheptulose-7-phosphate with the release of an inorganic phosphate. This marks as another irreversible step of this stage. At the next step, transketolase again acts on the substrates, sedoheptulose-7-phosphate which transfer two carbon to the another substrate, glyceraldehydes-3-phosphate converting them into two pentose sugar phosphates naming ribose-5-phosphate and xylulose-5-phosphate. Condensation of two carbon fragments into glyceraldehyde-3-phosphate is possible because of TPP acting as a cofactor in enzyme transketolase. Another enzyme, ribose-5-phosphate isomerase converts ribose-5-phosphate into ribulose-5-phosphate. Enzyme ribulose-5-phosphate kinase catalyzing an exergonic reaction by the use of ATP to convert ribulose-5-phosphate into ribulose-1, 5-bisphosphate regeneration it so that calvin cycle continues again (Figure 4.7). In this step, the phosphate anhydride bond in ATP is substituted for a phosphate ester bond in ribulose 1, 5-bisphosphate. Ribulose-1, 5-bisphosphate acts as a starting substrate for the calvin cycle and hence its renewal is essential. This whole pathway includes various enzymes involved from which some are unique to plastid and cytosol and some are shared between the organelles like isomerase, epimerase, kinase, etc.

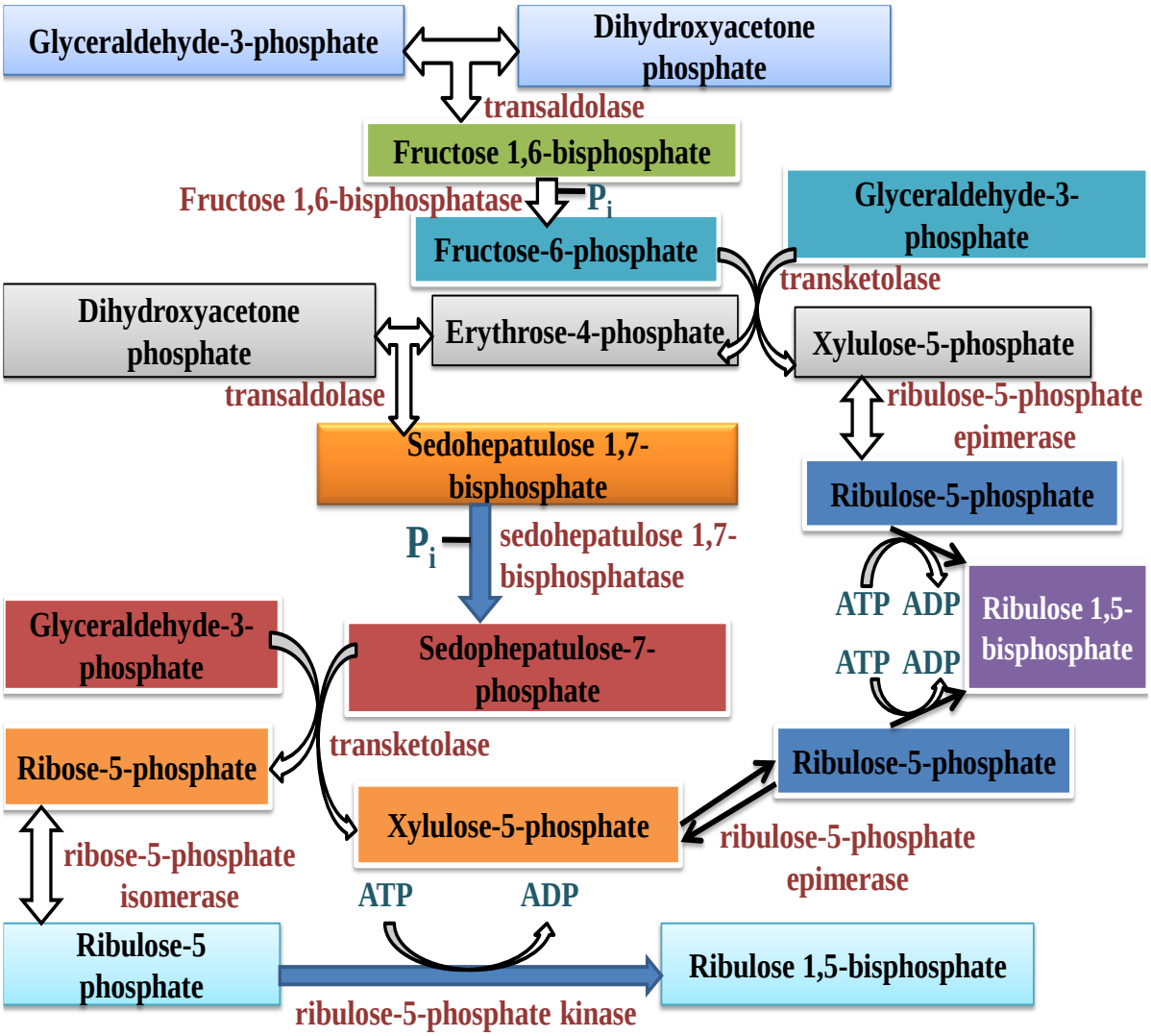

Figure 4.7: The third stage of Calvin cycle for regenerating ribulose 1, 5-bisphosphate.

The entire cycle involves three CO_2 molecules and one molecule of phosphate which on combining generate a molecule of triose phosphate. Three molecules of ribulose 1, 5-bisphosphate condenses with three molecules of CO_2 to generate six molecules of 3-phosphoglycerate which later on reduction produces six molecules of glyceraldehyde-3-phosphate through the expenditure of six molecules of ATP and six molecules of NADPH. In light dependent reactions, NADPH and ATP are produced in the same ratio (2:3) in photosynthesis as they are consumed in the Calvin cycle (Figure 4.8). For the production of one molecule of triose phosphate, 9 molecules of ATP are consumed releasing nine molecules of ADP and nine molecules of inorganic phosphate. Eight molecules out of nine molecules of inorganic phosphates condense with ADP to form ATP and the ninth molecule combines with triose phosphate. The remaining ninth molecule of ADP is converted to ATP via import of an inorganic phosphate from the cytosol to the plastid. The chloroplast stroma contains all the

necessary enzymes needed for the conversion of triose phosphates into starch during CO_2 assimilation but when not needed they are stored in the chloroplast as insoluble granules.

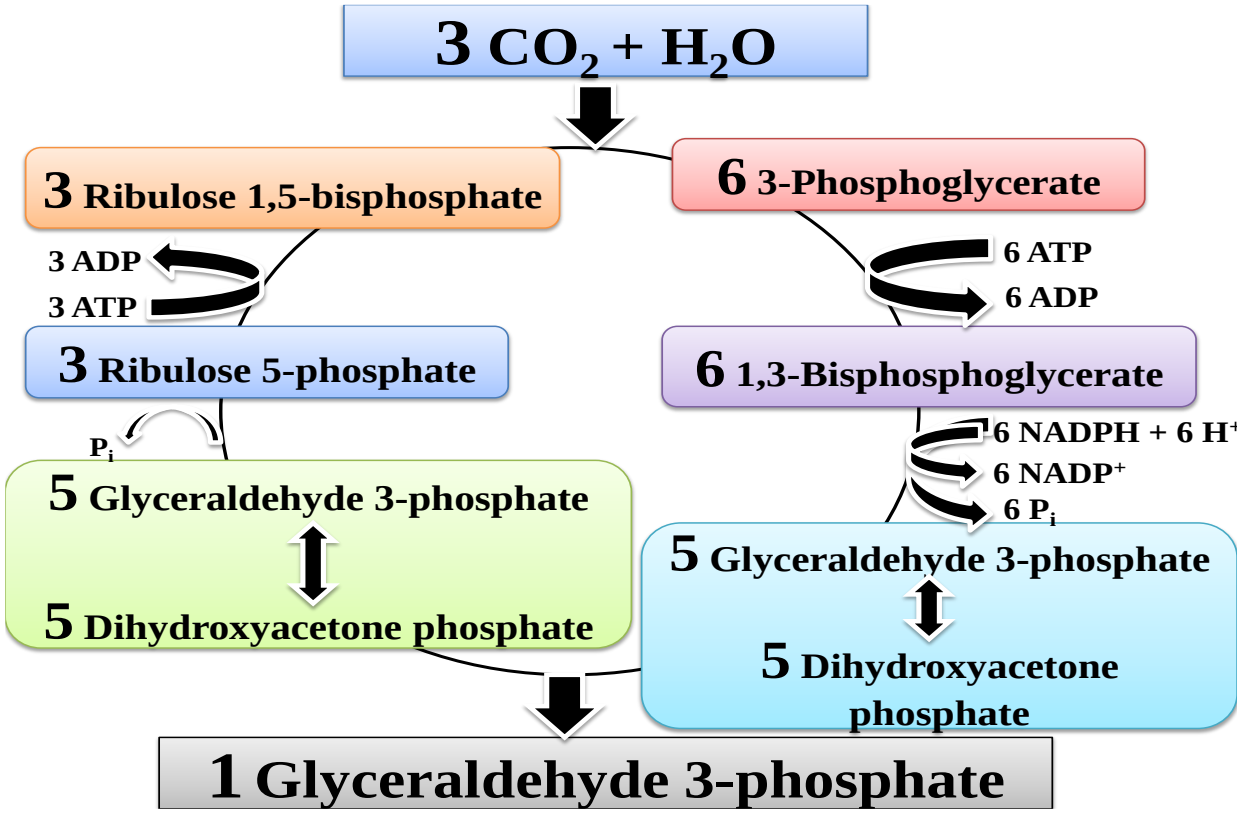

Figure 4.8: The CO_2 assimilation in the Calvin or C_3 cycle.

4.13 C_4 PLANTS

In C_3 plants, CO_2 is fixed in three carbon product, 3-phosphoglycerate but there is another process for CO_2 fixation temporarily through a four carbon product and the plants carrying out such process are called C_4 plants and the process of assimilating CO_2 in such a manner is called C_4 metabolism or the C_4 pathway (Figure 4.9). C_4 plants like maize, sorghum and sugarcane are generally grown in tropic or temperate zones with very high light intensity and high temperature. Due to increased light intensity, the natural process of photosynthesis increased at higher rates, there is a high growth rate, low photorespiration rates, less water loss and modified leaf structure which makes the plant specialized to thrive in the environment. CO_2 is first converted into HCO_3^- which acts as a substrate for phosphoenolpyruvate carboxylase (PEP carboxylase) in the cytosol of mesophyll cells instead of CO_2 resulting in the formation of four carbon product, oxaloacetate. The PEP carboxylase shows high affinity towards HCO_3^- and hence no competition is between the substrate as is seen in rubisco for CO_2 and O_2. The oxaloacetate undergoes reduction to form

malate by using NADPH and enzyme, malate dehydrogenase or through transamination, oxaloacetate is converted into aspartate. The malate or aspartate are transported into the neighboring cell of bundle-sheath through plasmodesmata which is a protein channel for transporting certain molecules between the cells and also act as a linkage between two cells. In the bundle-sheath cell, malic enzymes oxidize and decarboxylate malate into pyruvate, reducing $NADP^+$. In other cases, aspartate is transaminated into oxaloacetate and then reduced back to malate upon the release of CO_2 by malic enzyme or PEP carboxykinase. The pyruvate formed earlier through the decarboxylation of malate in the bundle sheath cells are transferred to mesophyll cells through plasmodesmata. Pyruvate is converted into PEP (phosphoenol pyruvate) by an unusual enzyme called pyruvate phosphate dikinase which causes simultaneous phosphorylation through one molecule of ATP from pyruvate to PEP and phosphate to pyrophosphate. The molecule of pyrophosphate is unstable and it is hydrolyzed to release two molecules of inorganic phosphate. The PEP carboxylation reaction causes release of CO_2 in the bundle sheath cells which results in local increase of high level of CO_2 and this high local concentration of CO_2 helps rubisco to perform its carboxylate activity and suppress oxygenase activity. CO_2 is then fixed in the form of 3-phosphoglycerate in the bundle sheath cells and the calvin cycle continues. Thus, in C_4 plants CO_2 assimilation takes place in the C4 pathway and starch and sugar is synthesized in bundle sheath cells through the C3 pathway. The C_4 pathway has a higher energy cost than the C_3 pathway. For every molecule of CO_2 assimilation, a molecule of PEP is needed on the cost of two high energy phosphate groups of ATP.

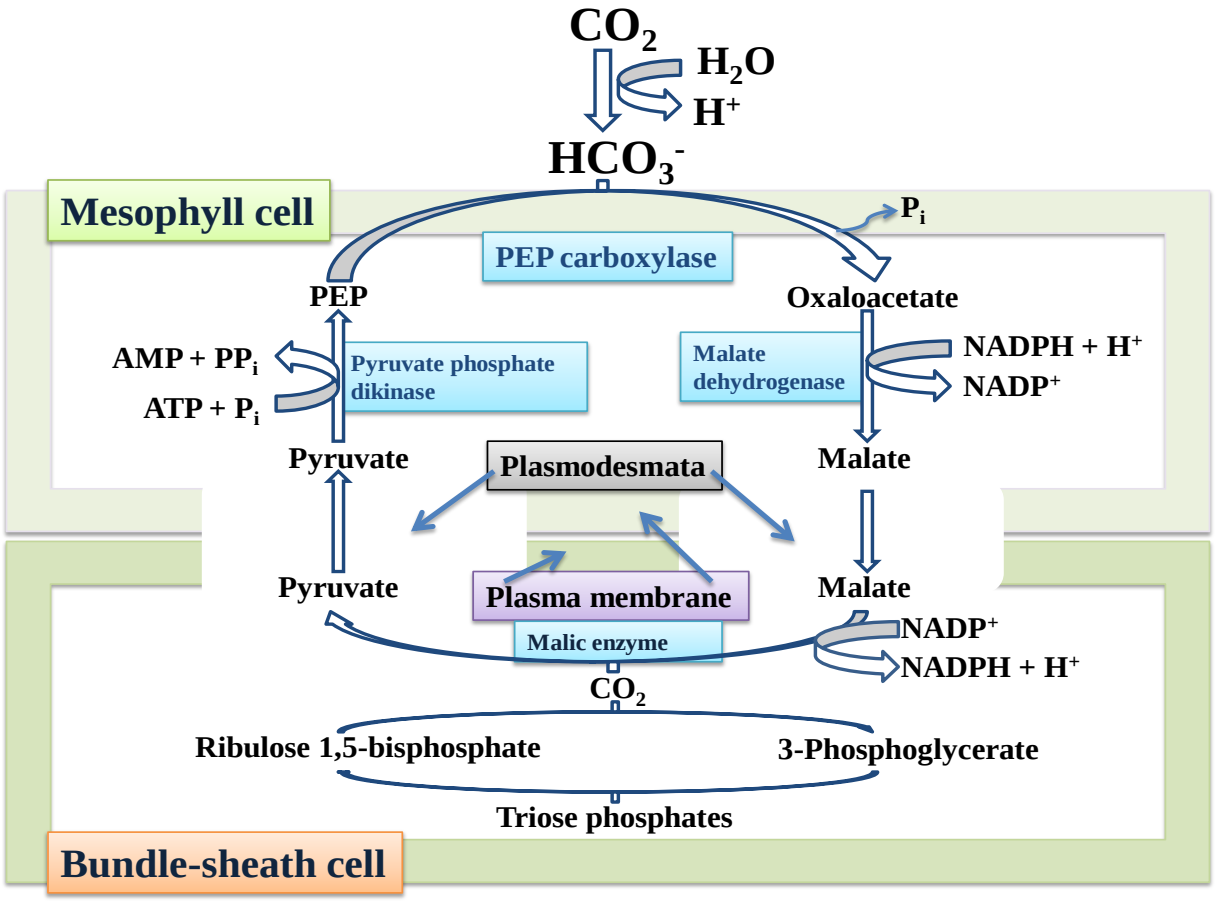

Figure 4.9: The Hatch-slack or C_4 pathway of CO_2 assimilation.

4.14 HATCH-SLACK PATHWAY

The oxygenase activity of rubisco increases with high temperatures and the plants thriving in hot climates have a threat to get prone to high rates of wasteful photorespiration. To solve such a problem, high local concentration of CO_2 at the site of the Calvin cycle in the photosynthetic cells maintains the overexploitation of photorespiration. This process was elucidated by M. D. Hatch and C. R. Slack. They revealed that four-carbon (C_4) compounds, oxaloacetate and malate are the carriers of CO_2 from mesophyll cells to the bundle-sheath cells (Figure 9). The high concentration of CO_2 in bundle sheath cells is maintained by decarboxylation of the four-carbon compound at the site of Calvin cycle whereas the three-carbon compounds return to mesophyll cells for another round of carboxylation. This process is distinctively called the C4 pathway and as it was firstly introduced by M. D. Hatch and C. R. Slack; it was also named Hatch-Slack pathway.

The overall reaction of this C_4 pathway is

CO_2 (in mesophyll cells) + ATP + H_2O $\longrightarrow$ CO_2 (in bundle-sheath cells) + AMP + 2 P_i + H^+

Two ATP molecules are consumed in CO_2 transportation to the chloroplasts of the bundle sheath cells through active transport driven by hydrolysis of one molecule of ATP into one molecule of AMP and two molecules of orthophosphate. In this way, CO_2 concentration is 20 times-fold greater in bundle-sheath cells than in the mesophyll cells. The C_4 pathway and calvin cycle operates simultaneously and the net reaction is given as

6 CO_2 + 30 ATP + 12 NADPH + 12 H_2O $\longrightarrow$ $C_6H_{12}O_6$ + 30 ADP + 30 P_i + 12 $NADP^+$ + 18 H^+

Upon each molecule of hexose, 30 molecules of ATP are consumed in the presence of C_4 pathway and in the absence; consumption of 18 molecules has been reported. For maintaining the high temperature in the bundle-sheath cells of C_4 plants, 12 molecules of ATP are consumed.

4.15 CAM PLANTS

Plants thriving in hot, dry and less availability of water are considered as succulent plants like cactus. Such plants have some modification for their CO_2 fixation within them. Due to high temperature at day time, plants do not cause the opening of stomata to prevent loss of water from plant tissues. Rather than the day time, stomata open at night time and CO_2 is allowed to enter when air is cooler and moisture. Entered CO_2 is converted into oxaloacetate by PEP carboxylase. Malate dehydrogenase reduces oxaloacetate into malate and it is stored in vacuoles due to its low pH which is not a favorable environment for cytosolic and plastid enzymes to function adequately. In this way, rubisco assimilates CO_2 and the calvin cycle continues. This method was discovered in stonecaps which belong to the perennial flowering plants of the Crassulaceae, it is named as crassulacean acid metabolism and the plants are called CAM plants.

4.16 FACTOR AFFECTING PHOTOSYNTHESIS

There are certainly many factors affecting the rate of photosynthesis which determines the complete physiology and functioning of plants. Among those factors, light intensity, carbon dioxide and temperature are the most important. The rate of photosynthesis rapidly decreases in the absence of photosynthesis. The presence of light does not positively sustain the process of photosynthesis if the environment lacks sufficient carbon dioxide. CO_2 acts as a limiting factor for the rate of photosynthesis when light is in abundance. Temperature is another factor playing a significant role in the photosynthesis rate. Certain modifications allow some modified plants to thrive in hot climates and they are either perennial or succulent. But in general plants do not thrive in too cold climates and too much temperature does not also support photosynthesis which

determines the importance of optimum temperature for best growth within plants. This optimum temperature is usually species specific.

4.17 PHOTORESPIRATION

Photorespiration is a cost effective side reaction of photosynthesis due to lack of specificity of the enzyme rubisco. CO_2 act as an absolute substrate for rubisco during the process of photorespiration other than molecular oxygen (O_2). O_2 and CO_2 competes with each other for the active site of rubisco and after three or four turnovers, rubisco catalyzes the reaction with O2 to produce 3-phosphoglycerate and 2-phosphoglycolate which is considered as an metabolically waste product. Photorespiration is the result of oxygenase activity of enzyme rubisco. This process does not contribute to any carbon fixation, lead to the production of wasteful product 2-phosphoglycolate and utilizes significant amounts of cellular energy upon the release of previously fixed CO_2. The O_2 and CO_2 solubility depends upon the temperature and it increases as temperature goes up. The affinity of rubisco for CO_2 decreases with increased temperature and enzyme's oxygenase activity becomes more pronounced leading it to proceed towards the wasteful oxygenase reaction. The wasteful product, 2-phosphoglycolate, undergoes through the glycolate pathway in the chloroplast and it is considered as a salvage pathway but is costly. A phosphatase enzyme converts 2-phosphoglycolate into glycolate and then it is transported into peroxisome where glcolate is oxidized in the presence of molecular oxygen to yield glyoxylate through the enzyme glycolic acid oxidase. A transamination reaction results in the production of glycine and it is transported to the mitochondrial matrix from the peroxisome where it undergoes oxidative carboxylation by the glycine decarboxylase complex and produces serine. The enzyme oxidizes glycine to CO_2 and NH_3 with the simultaneous reduction of NAD^+ into NADH and the remaining carbon fragment is transferred from glycine to the cofactor tetrahydrofolate. The serine hydroxymethyltransferase transfers one carbon unit from tetrahydrofolate to a second glycine and produces a serine molecule as a result. The net reaction by the glycine decarboxylase complex and serine hydroxymethyltransferase can be summarized as:

$$2\ \text{Glycine} + NAD^+ + H_2O \longrightarrow \text{serine} + CO_2 + NH_3 + NADH + H^+$$

The serine went on conversion and produces hydroxypyruvate and then glycerate upon the action of α-hydroxy acid reductase. The glycerate is again transported from peroxisome to chloroplast stroma and is converted into 3-phosphoglycerate as a final product. The 3-phosphoglycerate is now ready to enter the calvin cycle as it was originally destined for and the pathway is salvaged as a result (Figure 4.10). The combined reaction of rubisco activity and glycolate pathway contribute to photorespiration and this process is also called as C_2 cycle or the oxidative photosynthetic carbon cycle.

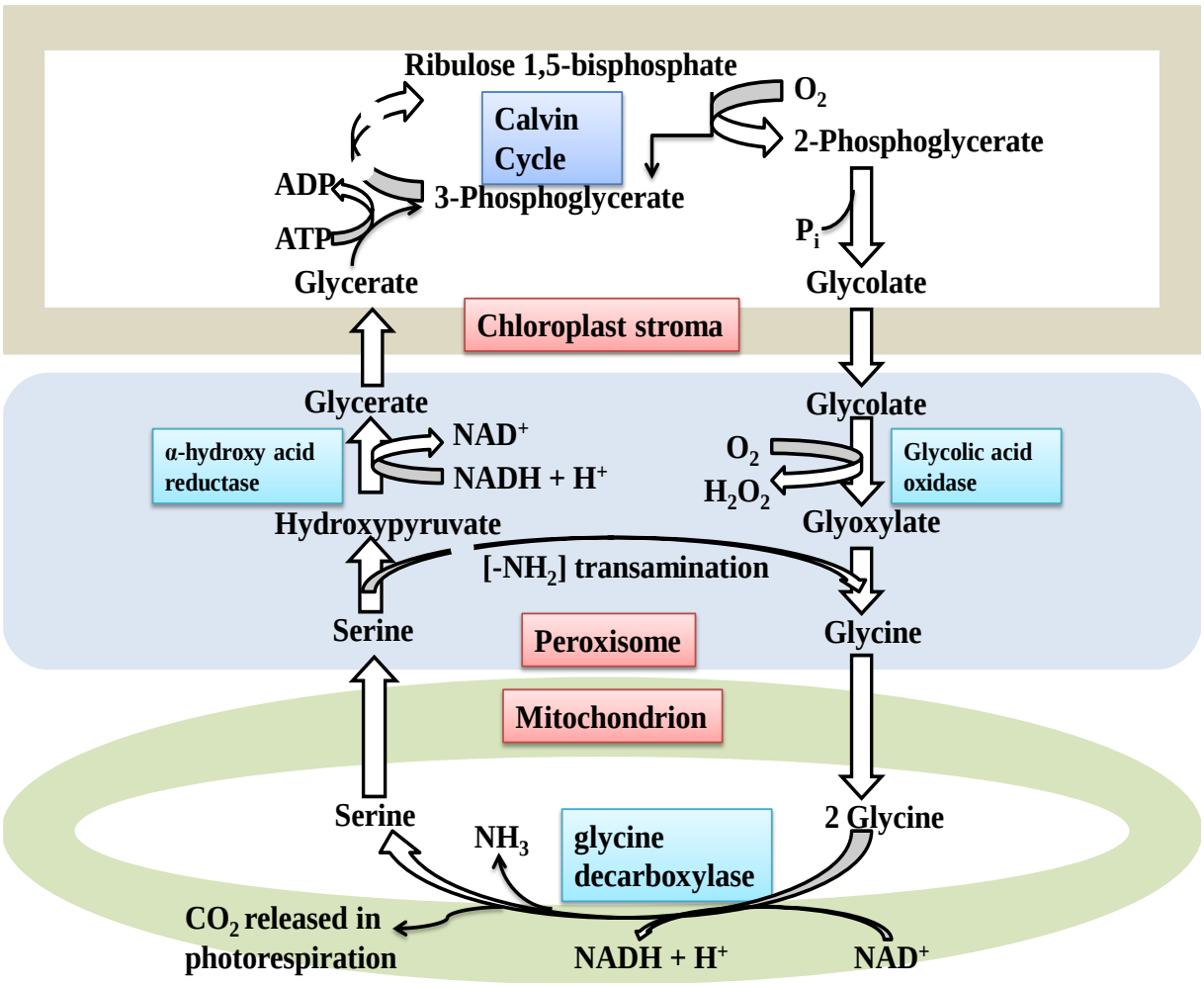

Figure 4.10: The salvage glycolate pathway for the production of 3-phosphoglycerate from the metabolic waste 2-phosphoglycolate.

4.18 SUMMARY

Photosynthesis is the process of reducing carbon in the presence of sunlight to yield carbohydrates as energy fuel directly for the photosynthetic organisms which produced them and indirectly for the non-photosynthetic organisms. The light energy is absorbed through the chlorophyll and accessory molecules to the reaction centre to trap energy for exciting electrons from ground state to excited state and transferred to other acceptor molecules. Chlorophyll and accessory photosynthetic pigments like carotenoids, phycoerythrin, etc. absorb light energy at different wavelengths to harness energy for electron transport within the photosystem I and II. When electrons flow through the both photosystem (photosystem II with reaction center P680 and photosystem I with reaction center P700) then Z-scheme is formed and non-cyclic electron flow is generated but when only photosystem I with reaction center P700 is involved for electron flow due to flow back of electrons from cytochrome b_6f complex so that electrons flow back to

the photosystem I, then the flow is called cyclic electron flow. The chloroplast is divided into stroma and lumen region and when due to the proton transport, concentration of protons is increased in the lumen side then an electrochemical gradient is produced. In plants, proton pumping across the thylakoid membrane produces proton driven forces which to generate ATP through the help of enzyme complex ATP synthase which is made up of two functional subunits which are CF_0, a transmembrane and CF_1, a peripheral membrane protein of the chloroplast. The calvin cycle fixes CO_2 into three carbon products 3-phosphoglycerate. Its production is responsible for naming the plant as C_3 plants. Ribulose 1, 5-bisphosphate carboxylase/oxygenase (Rubisco) is the enzyme with carboxylase activity is responsible for catalyzing the CO_2 incorporation into organic form but oxygenase activity of rubisco leads to the process of photorespiration in which a metabolically waste product called 2-phosphoglycolate is formed in the dark. The glycolate pathway converts 2-phosphoglycolate into 3-phosphoglycerate by which it can enter the calvin cycle. There is another group of plants which produces the four-carbon compound, oxaloacetate or malate to fix CO_2 and such plants are known as C_4 plants.

4.19 GLOSSARY

Accessory pigments: They are visible light-absorbing pigments which trap light energy.

CAM plants: These are succulent plants which fix CO_2 as oxaloacetate in the dark.

Carbon-assimilation reaction: It is a reaction in which CO_2 is converted into organic compounds.

Carbon fixation reaction: It is a reaction catalyzed by rubisco which incorporates CO_2 into organic compounds.

Cyclic electron flow: The electron flow through the photosystem I cause a cyclic pathway in the light-inducing fashion.

Cyclic photophosphorylation: It is the process of ATP synthesis through the cyclic electron flow in photosystem I.

C_4 plants: these are the plants in which CO_2 is fixed as four carbon compounds in the form of oxaloacetate or malate before the action of rubisco to enter the calvin cycle.

Glycolate pathway: It is a metabolic pathway in which waste product, 2-phosphoglycolate produced during photorespiration is converted into 3-phosphoglycerate.

Non-cyclic electron flow: It is the electro flow path through photosystem I and II in which electrons flow from water to $NADP^+$ in the oxygen-evolving process of photosynthesis.

Photophosphorylation: It is the light driven process in which electrons are transferred in photosynthetic cells and ATP is generated.

Photorespiration: It is a process which occurs in plants living in high temperature zones due to the oxygenase activity of enzyme rubisco causing the oxidation of phosphoglycolate.

Photosystem: An array of photosynthetic molecules with accessory molecules which absorb photons and transfer light energy to the reaction centre.

Photosynthesis: It is the process of fuel generation in the form of organic compounds in the presence of light energy by utilizing carbon dioxide and water as a reducing agent.

Plastids: It is self-replicating organelle present in plants which can differentiate into chloroplast or amyloplast.

4.20 SELF ASSESSMENT QUESTIONS

4.20.1 Fill in the blanks

1. _____ acts as a starting material for the calvin cycle to initiate.
2. Stacked region of chloroplast contains photosystem _____.
3. Unstacked region of chloroplast contains _____ and _____.
4. ____ is the enzyme which catalyze CO_2 assimilation into organic form in calvin cycle
5. _____ are the total protons moved from stroma to the thylakoid membrane in the Z-scheme of electron transport.
6. Albert Frankel discovered the process of light-dependent ATP production in membranous structures called _____ derived from photosynthetic bacteria.
7. The reaction centre of photosystem I is _____.
8. In the absence of _____, plants are threatened and quickly killed.
9. The calvin cycle occurs in the _____ of chloroplasts.
10. Due to the oxygenase activity of rubisco ____ is formed as a metabolically wasteful product.

Answer Key:

1. Ribulose-1, 5-bisphosphate
2. II
3. Photosystem I, ATP synthase
4. Ribulose 1, 5-bisphosphate carboxylase/oxygenase (Rubisco)
5. 12 H^+
6. Chromatophores
7. P700

8. Carotenoids
9. Stroma
10. 2-phosphoglycolate

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4.23 TERMINAL QUESTIONS

1. Differentiate between cyclic and noncyclic photophosphorylation.
2. With the help of a suitable diagram, an elaborate Z-scheme of electron flow.
3. Describe the process of photorespiration and the need of the glycolate pathway.
4. What is the role of different photosynthetic pigments in the process of photosynthesis?
5. Explain the factors affecting the photosynthesis process.

UNIT-5- RESPIRATION

Contents:

5.1OBJECTIVES

- To study the respiration and its types.
- To study the Fermentation
- To study the Electron transport chain
- To study the ATP synthesis
- To study the Energy transfer and conservation

5.2 INTRODUCTION

Respiration is the most important process of all the living beings in which oxygen enters into the cells and oxidizes various food substances like carbohydrates, fats and proteins present in them. As a result of oxidation, energy is produced which is utilized in various physiological and synthetic processes. In the very beginning the term respiration was used for the respiratory movements of animals but later on it included all the energy producing processes. The plants possess some different type of respiration as:

- The plants lack respiratory system and respiratory movements
- The exchange of gases in plants in also different because during day light respiration is slightly suppressed due to photosynthesis
- Sometimes the plants do not use oxygen during respiration (anaerobic)
- In some cases CO_2 is not liberated outside the plant cells.

The energy stored in carbohydrate molecules during photosynthesis is released during **cellular oxidation** of carbohydrates into CO_2 and H_2O. This is called as **Respiration.** In respiration the oxidation of various organic food substances carbohydrates, fats, proteins *etc.*, may take place. Among these **glucose** is commonest. Its oxidation proceeds as is shown in the following equation.

$$\mathbf{C_6H_{12}O_6 + 6O_2 \rightarrow 6H_2O + Energy\ (686\ K.\ Cal)}$$

This oxidation is not so simple and does not take place in one step. Breakdown of glucose involves many steps releasing energy in the form of **ATP** molecules and forming a number of carbon compounds **(Intermediates)** in a very well organized sequence.

History of Respiration

- The term “respiration” was used in the beginning of fifteenth century but is importance was worked out by Crooke (1615). Initially, it is believed that respiration is found only in animals and not in plants.
- Malpighi (1619) demonstrated that oxygen is required in high amount during germination of seeds.

- Sheele (1777) told that during respiration seeds take O_2 inside and liberate CO_2.
- Lavoisier demonstrated that respiration is a combustion process in which the energy is produced in the same way as is produced by during the coal but both the processes are different.
- Ingen-Housz (1779) concluded on the basis of his experiments that all plants liberate CO_2 in dark.
- De Saussure (1804 and 1822) measured the amount of O_2 taken and CO_2 liberated by plants. Sachs (1864) told that the growth is directly proportional to the rate of respiration.
- Sachs (1865) told that exchange of gases is related with two main processes, of which one is now called photosynthesis and other as respiration.
- Pasteur (1870) studied the fermentation by yeast cells and demonstrated that alcohol is formed as a result of fermentation and anaerobic respiration.
- Mac Munn (1886) discovered histohaematin (cytochromes).
- Bach (1901) confirmed that plant extracts can oxidize phenols in presence of O_2.
- Pfeffer (1900) proved that the first stage in aerobic and anaerobic respiration both is similar.

5.3 RESPIRATION

Respiration is the process of gaseous exchange where O_2 is taken in and CO_2 is liberated outside. Hackett (1959) called respiration 'life with air'. According to him, respiration is a complex process which includes following processes:

(i) Oxidation and fragmentation of organic compounds.
(ii) Transfer of electrons which ultimately form water by the union of hydrogen and oxygen.
(iii) Liberation of energy which is utilized in various physiological processes.

According to stiles and Leach (1960), the respiration is a complex process where complex compounds are oxidized into simpler compounds and energy is released.
Respiration is completed into two parts.

- The first part where carbohydrates etc. are converted into pyruvic acid is called glycolysis.
- The second part where pyruvic acid is oxidised into CO_2 and H_2O is called Kreb's Cycle.

Types of Respiration

Respiration is mainly of two types: (i) Anaerobic respiration, (ii) Aerobic respiration

5.3.1 Anaerobic Respiration

Respiration occurring in absence of oxygen is called *anaerobic respiration.* It is found only in certain plants. During anaerobic respiration, the food materials like carbohydrates, fats and proteins are incompletely oxidized resulting in the formation of alcohol and CO_2. The energy

released during anaerobic respiration is quite less (about 21 k.cal. per molecule) in comparison to aerobic respiration (about 673 k.cal./mol.). Of the various end products, CO_2 is liberated in the atmosphere, alcohol is retained inside the cells and energy is utilized only in a few processes. The alcohol may be deoxidized by aerobic respiration.

$$\mathbf{C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + 21\ k.\ cal.}$$

Glucose alcohol energy

Anaerobic respiration occurs during resting stage of seeds, initial stages of seed germination in the cells of fruit walls, several micro-organisms and *Ascaris* etc.

5.3.2 Aerobic Respiration

Respiration occurring in presence of oxygen is called *aerobic respiration.* In most of the plants usually aerobic respiration is found which is stopped in absence of O_2. During aerobic respiration, the food materials like carbohydrates, fats and proteins are completely oxidized into CO_2 and H_2O and large amount of energy is released. The oxidation of one molecule of glucose produces about 673 k. calories of energy. Of the various end products, CO_2 is liberated into atmosphere, water is retained inside the plant cells and energy is utilized in various biological and synthetic processes. Aerobic respiration can be shown by following equation.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H2O + 673.\ K.\ Cal.$$

Glucose Oxygen Carbon dioxide Water Energy

The produced energy is absorbed by ADP and is stored in the form of ATP. The energy is again produced by the hydrolysis of ATP, when required. Although, aerobic respiration has been shown above by a single equation but it is a very complex process and is completed in several steps. The whole process may be divided into two parts:

(a) Conversion of glucose into pyruvic acid (glycolysis).
(b) Aerobic oxidation of pyruvic acid into CO_2 and H_2O (oxidative decarboxylation and Kreb's cycle).

Kostychev demonstrated in *Aspergillus niger* that during aerobic respiration the oxidation of lactic acid, glycerol and mannitol takes place. During oxidation of peptones, amino-acids are formed first which are then oxidized into CO_2 and H_2O.

(a) Glycolysis

The steps of glycolysis were first studied by three German scientists, Embden, Meyerhof and Parnas and therefore, can be named as E.M.P. pathway. It can take place even in the absence of O_2. In glycolysis, 6 carbon compound (glucose) is broken down through a series of reversible

enzymic reaction into 3-carbon compound the pyruvic acid (a triose) in cytoplasm. It is divided into four stages:

1. Synthesis of Fructose 1, 6- diphosphate
2. Cleavage of Fructose 1, 6- diphosphate
3. Oxidation of 3-Phosphoglyceraldehyde
4. Synthesis of Pyruvic acid.

Various stages of glycolysis are as follows:

Stage 1: Synthesis of Fructose 1, 6- diphosphate

1. Glucose molecule reacts with ATP molecule to form glucose-6-phosphate (Robinson's ester). Enzyme: *Hexokinase*
2. Glucose 6-Phosphate is isomerized into Fructose 6- Phosphate (Newberg's ester) Enzyme: *Phosphohexose isomerase.*
3. Fructose 6- Phosphate reacts with one molecule of ATP to form Fructose 1, 6-diphosphate (Hardern and Young's ester)**.** Enzyme: *phosphohexose kinase* and cofactor Mg^{2+}

Stage 2: Cleavage of Fructose 1, 6- diphosphate

4. Fructose 1, 6 -Diphosphate is converted into two **trioses 3-Phosphoglyceraldehyde and Dihydroxy acetone phosphate.** Enzyme: Aldolase.

   The two trioses may isomerizes into each other in the presence of *phosphotriose isomerase* so that a balance is maintained.

Stage 3: Oxidation of 3-Phosphoglyceraldehyde

5. 3-Phosphoglyceraldehyde reacts with H_3PO_4 and forms 1, 3- diphosphoglyceraldehyde. The reaction is non-enzymatic.
6. 1, 3-Diphosphoglyceraldehyde is oxidized to form 1, 3-diphosphoglyceric acid. Enzyme: Triose-phosphate dehydrogenase and cofactor NAD.

   NAD is reduced to $NADH_2$. This reaction is inhibited by M/1000 conc. lodoacetate.
7. 1, 3- Diphosphoglyceric acid reacts with ADP to form one mol. of ATP and 3-Phosphoglyceric acid. Enzyme: Phosphoglyceric transphosphorylase

Stage 3: Oxidation of 3-Phosphoglyceraldehyde

8. 3-Phosphoglyceric acid is isomerised into 2-Phosphoglyceric acid.

Enzyme: *Phosphoglyceromutase.*

9. 2-Phosphoglyceric acid is converted into 2-Phosphoenol-pyruvic acid.

   Enzyme: Enolase. The reaction is inhibited by M/40 conc. of NaF.

10. 2-Phosphoenol pyruvic acid reacts with ADP to form one molecule each of ATP and Pyruvic acid. Enzyme: Pyruvate kinase

The carboxylic group of the pyruvic acid is derived from carbon no. 4 or 5 of the glucose molecule. Glycolysis or EMP pathway is common in both aerobic and anaerobic respiration. There is gain of 2ATP molecules per hexose sugar molecule oxidized during this process. These ATP molecules are produced by substrate level phosphorylation.

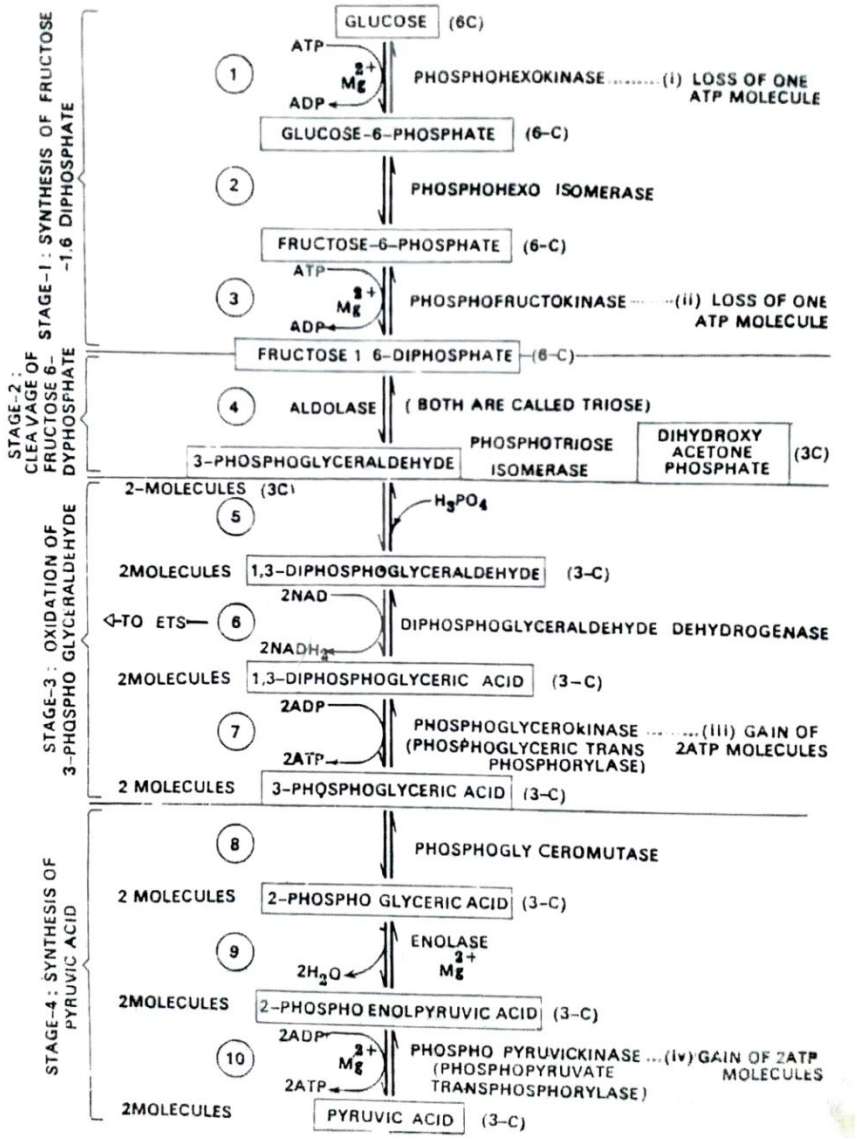

Fig.:5.1. Stages in Glycolysis

Summary of Glycolysis

- In glycolysis, from one molecule of glucose, two molecules of pyruvic are formed.
- In this, four molecules of ATP are formed (2 ATP at stage 3, step 7 + 2ATP at stage 4, step 10). Because two molecules of ATP are consumed in phosphorylation reactions (1 ATP at stage 1, step 1 + 1 ATP at stage 1, step 3 = 2 ATP molecules), therefore, during glycolysis there is a net gain of 2 ATP molecules (4 ATP — 2ATP = 2 ATP).
- Two molecules of NAD are reduced to two molecules of $NADH_2$ (stage 3, step 6) which later on oxidised aerobically to yield six molecules of ATP (one NAD molecule after oxidation produces 3 molecules of ATP). Thus, the total gain of ATP molecules during glycolysis in presence of oxygen will be increased to eight instead of two.
- The energy of glucose becomes stored partly in ATP molecules and partly in $NADH_2$ molecules.
- Oxygen is not required during glycolysis.
- In glycolysis, CO_2 is also not produced.

(b) Aerobic oxidation of pyruvic acid into CO_2 and H_2O (Oxidative decarboxylation and Kreb's cycle).

The oxidation of pyruvic acid (3-carbon compound) in aerobic conditions takes place through tricarboxylic acid cycle (TCA Cycle) discovered by Sir Hans Kreb., The pyruvic acid before entering into Kreb's Cycle is first converted into 2-carbon compound, acetyl coenzyme-A. from 3-carbon compound with the help of several enzymes and coenzymes.. During this process, one molecule of CO_2 is liberated from pyruvic acid. This mechanism or process is called oxidative decarbaxylation. It takes place in the mitochondria of aerobic cells. **Oxidative Decarboxylation:**

Oxidative decarboxylation is completed in several steps catalysed by an enzyme complex pyruvate dehydrogenase. The complex includes following enzymes and coenzymes. **Enzymes:** Pyruvic acid decarboxylase, Dihydroxy Lipoyl transacetylase, Dihydrolipoyl de-hydrogenase. **Coenzymes:** Thiamine pyrophosphate (TPP), Lipoic acid, Coenzyme A, and NAD.

Different steps of oxidative decarboxylation are as follows:

1. Pyruvic acid is converted to form acetaldehyde. One molecule of CO_2 is evolved.
Enzyme: *Pyruvic acid decarboxylase.*

2. Acetaldehyde (acetyl group) reacts with coenzyme Thiamine pyrophosphate (TPP) to form active acetaldehyde.

3. Active acetaldehyde after combining with lipoic acid forms acetyl lipoic acid.
Enzyme: *Dihydroxylipoyl transacetylase.*

4. Acetyl lipoic acid reacts with Coenzyme A resulting into formation of acetyl CoA and reduced lipoic acid.
Enzyme: *Dihvdrolipoyl dehydrogenase.*

5. Reduced lipoic acid after reacting with NAD is oxidized and NAD is reduced to $NADH_2$.
Enzyme: *Lipoate dehydrogenase*
The sum of all the above reactions is as follows:

$$\text{Pyruvic acid} + \text{Coenzyme A} + \text{NAD} \longrightarrow \text{Acetyl CoA} + CO_2 + NADH_2.$$

The $NADH_2$ in the above equation combines with half molecule of oxygen to form one molecule of water. In this process, three molecules of ADP are also oxidized resulting into formation of three molecules of ATP.

$$NADH_2 + 1/2O + 3ADP \longrightarrow NAD^+ + H_2O + 3ATP$$

Summary of Oxidative Decarboxylation

- Two molecules of molecules of pyruvic acid form two molecules of acetyl CoA, CO_2, and $NADH_2$ each.
- Two molecules of $NADH_2$, after being oxidized produce 6 molecules of ATP.
- Oxidative decarboxylation acts as a link between glycolysis and Kreb's Cycle.

Kreb's Cycle or TCA Cycle or Citric Acid Cycle

An English biochemist, Sir H..A. Kreb's (1937), discovered this cycle for the first time in nematodes. After his name, the cycle was called Kreb's cycle. He was awarded Nobel Prize for this discovery. All the reactions of Kreb's cycle takes place inside the mitochondria and through this pyruvic acid, fatty acids, fats and amino acids are oxidized into CO_2 and water. Thus Kreb's cycle represents the common path for the metabolism of carbohydrates, fats and protein.

Acetyl CoA formed during oxidative decarboxylation enters into Kreb's cycle for further oxidation.

The different steps of Kreb's cycle are as follows:

1.Acetyl-CoA condenses with oxaloacetic acid in the presence of condensing enzyme and water molecule to form citric acid. CoA becomes free. Citric acid is degraded step wise until oxaloacetic acid is regenerated.

Enzyme: *Citrate synthetase*

2. Citric acid is dehydrated to from **Cis-Aconitic Acid.**

Enzyme: *Aconitase*

3. Cis-Aconitic Acid reacts with one mole of water to from **Isocitric Acid.**

Enzyme: *Aconitase*

4. Iso-Citric Acid is oxidized to **Oxalosuccinic acid**. Coenzyme-II *i.e.*, **NADP is reduced** in the reaction.

Enzyme: *Isocitric-dehydrogenase*

5. Oxalosuccinic acid is **decarboxylated** to form **a-ketoglutaric acid.** A second molecule of CO_2 is released.

Enzyme: *Oxalosuccinic decarboxylase*

6. α-Ketoglutaric acid reacts with CoA and NAD and is **oxidatively decarboxylated** to form **Succinyl CoA** and a third mol. of **CO_2** *i*s **released. NAD is reduced** in the reaction which is **irreversible.** The enzyme complex involved here is very much similar to the *pyruvate dehydrogenase complex.*

Enzyme: α -*ketoglutaric acid-dehydrogenase* complex

7. Succinyl CoA reacts with water molecule to form **Succinic acid.** CoA becomes free and one mole of GDP (Guanosine diphosphate) is phosphorylated in presence of inorganic phosphate to form one molecule of GTP**. GTP again reacts with ADP to form ATP and GDP.**

Enzyme: *Succinic thiokinase*

8. Succinic acid is oxidized to **Fumaric acid**. Coenzyme **FAD** (Flavin Adenine Dinucleotide) is **reduced** to $FADH_2$.

Enzyme: *Succinic dehydrogenase*

9. One molecule of H_2O is added to Fumaric acid to form **Malic acid.**

Enzyme: *Fumarase*

10. In the last step Malic acid is oxidized to oxaloacetic acid . One molecule of coenzyme-I, *i.e.*, **NAD** is reduced to $NADH_2$.

Enzyme: *Malic- dehydrogenase*

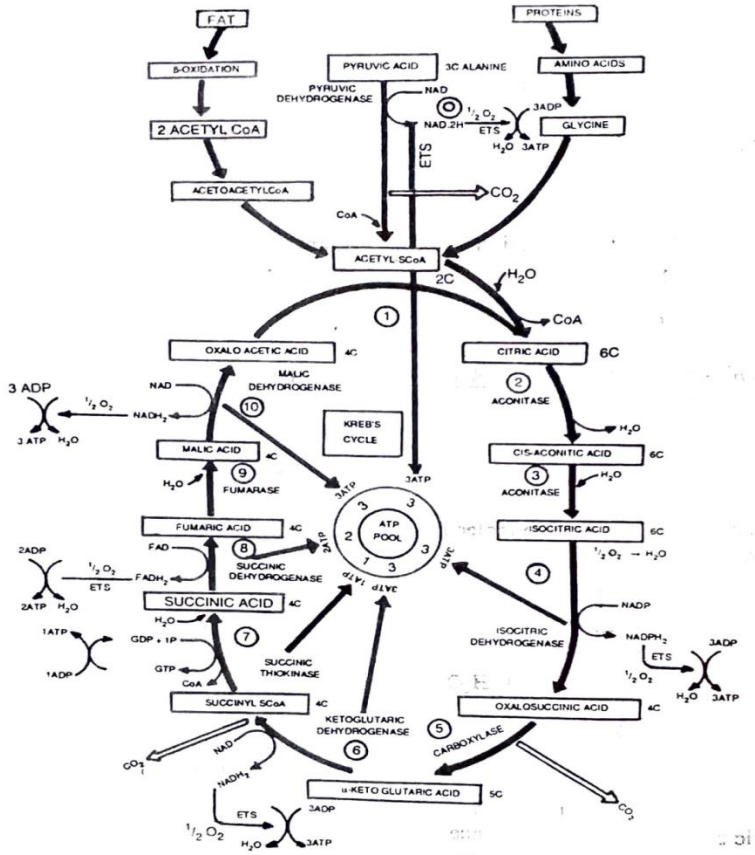

Fig.: 5.2. Steps in Oxidative decarboxylation and Kreb's cycle

Thus, as a result of oxidation of pyruvic acid one molecule of CO_2 in oxidative decarboxylation and two molecules of CO_2 in Kreb's cycle (steps 5 and 6) are liberated. The total number of CO_2 evolved becomes three which indicate that the 3-carbon pyruvic acid has been completely oxidized. Now, because two molecules of pyruvic acid which are formed by one molecule of glucose in glycolysis, enter into Kreb's cycle for oxidation, a total of 6 CO_2 molecules will be evolved. All the $NADH_2$ and $FADH_2$ molecules synthesized during glycolysis and Kreb's enter into electron transport system for oxidation where they are oxidized in presence of oxygen to produce e ATP molecules. The complete oxidation of one molecule of glucose produces 38 ATP molecules of which 8 ATP molecules are produced in glycolysis., 6 ATP molecules in oxidative decarboxylation and 24 ATP molecules in Kreb's cycle.

The summary of aerobic oxidation of glucose is as follows:

i. Glucose $\longrightarrow$ 2 Pyruvic acid + $2H_2$ (Glycolysis) 8 ATP

ii. Pyruvic acid + 2 CoA.SH $\longrightarrow$ 2 Acetyl.CoA + $2H_2$ + $2CO_2$ 6 ATP
(oxidative decarboxylation) (in 2 turns)

iii. Acetyl.CoA + 6 H2O $\longrightarrow$ 2CoA.SH + $8H_2$ + 4 CO_2 24 ATP
(Kreb's cycle) (in 2 Turns)

Sum of reactions (i), (ii) & (iii):

Glucose + $6H_2O$ $\longrightarrow$ $6CO_2$ + $12H_2$ + 38ATP (iv)

$12H_2 + 6O_2 \longrightarrow 12H_2O$ (v)

Sum of reactions (iv) and (v):

Glucose + $6O_2 \longrightarrow$ 6CO2 + $6H_2O$ + 38ATP

5.4 FERMENTATION

It is a type of anaerobic respiration where the substrates are incompletely oxidized in absence of oxygen. The glucose is incompletely oxidized into CO_2 and ethyl alcohol (C_2H_5OH) during fermentation. Sometimes instead of alcohol, organic acids like lactic acid, acetic acid, butyric acid, oxalic acid or citric acid are formed.

Based upon the type of end products produced during incomplete oxidation of glucose, commonly following types of fermentation are found.

Alcoholic Fermentation

In this type, end product is ethyl alcohol. During this process, glucose is converted into pyruvic acid which in turn converted into ethyl alcohol and CO_2. Intermediate product of alcoholic fermentation is acetaldehyde.

Formation of alcohol from pyruvic acid is completed in two steps:

1. The pyruvic acid is first decarboxylated to acetaldehyde in the presence of enzyme pyruvic decarboxylase.

   Pyruvic decarboxylase

   $CH_3.CO.COOH \longrightarrow CH_3CHO + CO_2$

   Pyruvic acid Acetaldehyde

2. Acetaldehyde is then reduced to ethyl alcohol by the enzyme alcohol dehydrogenase. One molecule of NADH is oxidized in presence of H+ into NAD.

   Alcohol dehydrogenase

   $CH_3CHO + NADH + H^+ \longrightarrow C_2H_5OH + NAD^+$

   Acetaldehyde Ethyl alcohol

The overall equation for anaerobic respiration involving alcoholic fermentation is as follows:

Zymase Complex

$C_6H_{12}O_6 \longrightarrow 2\ C_2H_5OH + 2\ CO_2 + 21$ k.cal.

Glucose (Yeast) Ethyl alcohol

Lactic Acid Fermentation

Here, the pyruvic acid is converted into **Lactic acid** by the enzyme *Lactic-dehydrogenase.* Coenzyme $NADH_2$ (produced in glycolysis) is oxidized.

It takes place in presence of Bacterium lactic acidi and Bacterium acidi lactici which convert the milk sugar into lactic acid. It is completed in following steps:

$$C_{12}H_{22}O_{11} + H_2O \longrightarrow C_6H_{12}O_6 + C_6H_{12}O_6$$

Lactose Glucose Galactose

The hexose sugar is converted into lactic acid in presence of Bacterium acidi lactici.

$$C_6H_{12}O_6 \xrightarrow{\text{Bacterium acidi lactic}} 2\ C_3H_6O_3$$

Hexose Lactic acid

The hexose sugar is converted into lactic acid and ethyl alcohol in presence of Bacterium lactic acidi. CO2 is also liberated.

$$C_6H_{12}O_6 \xrightarrow{\text{Bacterium lactic acidi}} C_3H_6O_3 + C_2H_5OH + CO_2$$

Glucose Lactic acid Ethyl alcohol

Acetic Acid Fermentation

In this type, glucose is converted into acetic acid. It takes place in presence of Acetobacter aceti and atmospheric O_2.

It is completed in two steps:

1. Glucose is converted into Ethyl alcohol.

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2\ CO_2$$

Glucose Ethyl alcohol

2. Ethyl alcohol is oxidised into acetic acid in presence of oxygen.

$$C_2H_5OH + O_2 \xrightarrow{\text{Acetobacter aceti}} CH_3COOH + H_2O + 118.2\ \text{k.cal.}$$

Ethyl alcohol Acetic acid

Butyric Acid Fermentation

In this fermentation, end product is butyric acid. It takes place in presence of *Clostridium butyricum* and *Bacillus butyricus* bacteria. In this hexose sugars and lactic acid is converted into butyric acid.

$C_6H_{12}O_6 \longrightarrow C_4H_8O_2 + 2H_2 + 2CO_2$
Hexose Butyric acid
$2C_3H_6O_3 \longrightarrow C_4H_8O_2 + 2H_2 + 2CO_2$
Lactic acid Butyric acid

5.5 ELECTRON TRANSPORT CHAIN

The electron transport chain is functioning inside the mitochondria. The mitochondrion is a subcellular organelle having the outer and inner membranes enclosing the matrix (Fig. 5.3, 5.5). The inner membrane is highly selective in its permeability characteristics. So, there are many protein systems, to transport specific molecules in and out of the mitochondrial membrane. Some important transporters are shown in Figure 5.4. Enzymes are specifically localised in mitochondria.

Location of Enzymes in Mitochondria:

The location of different enzymes in mitochondria is as follows:

Outer membrane:

- Mono amino oxidase
- Acyl Co synthetase
- Phospholopase A_2

In between outer and inner membranes:

- Adenylate kinase
- Creatine kinase

Inner membrane, outer surface:

- Glycerol-3-phosphatedehydrogenase

Inner membrane, inner surface:

- Succinate dehydrogenase
- Enzymes of respiratory chain

Soluble matrix

- Enzymes of citric acid cycle
- Enzymes of beta oxidation of fatty acid

The inner membrane contains the respiratory chain and translocating systems. The knobs like protrusions represent the ATP synthase system (Fig.5.5).

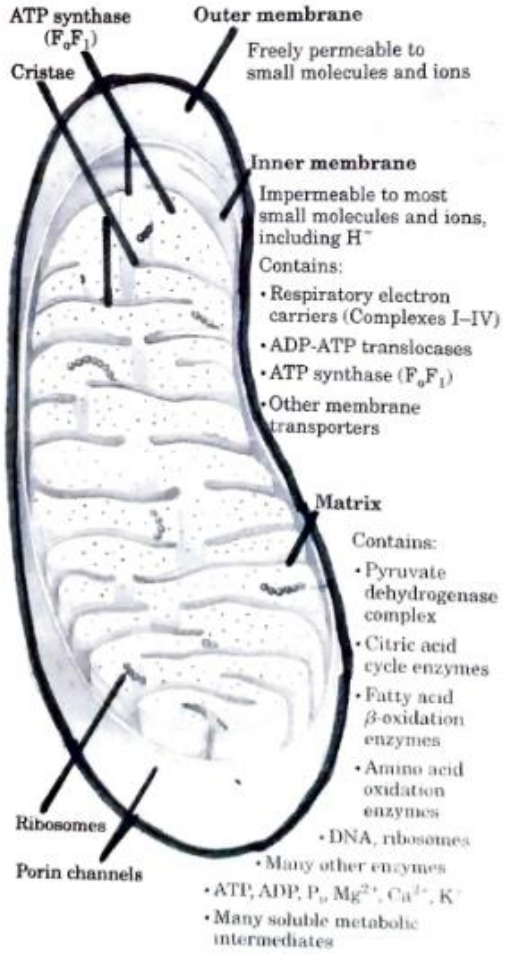

Fig:5.3. Biochemical anatomy of mitochondria

The conyolutions (cristae) of the inner membrane provide a very large surface area. The inner membrane of a single liver mitochondrion may have over 10,000 sets of electron-transfer systems (respiratory chains) and ALP synthase molecules, distributed over the membrane surface. Heart mitochondria, which have more profuse cristae and thus a much larger area of inner membrane, contain over three times as many sets of electron-transfer systems as liver mitochondria. The mitochondrial pool of coenzymes and inter-mediates is functionally separate from the cytosolic pool. The mitochondria of invertebrates, plants, and microbial eukaryotes are similar to those shown here, but with much variation in size, shape, and degree of convolutions of the inner membrane.

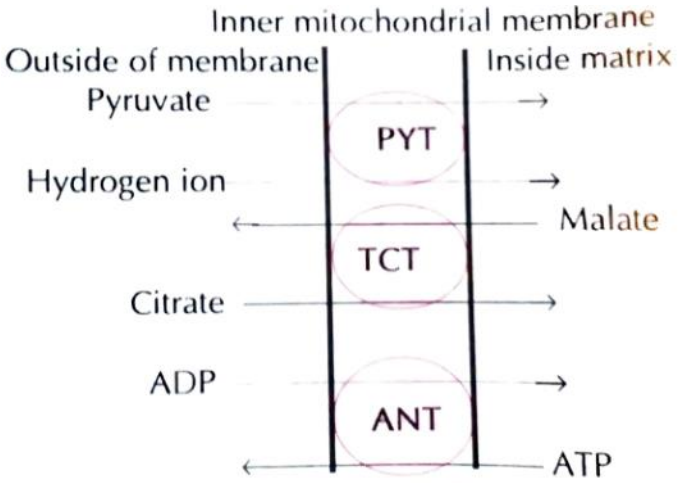

Fig: 5.4. Some Important Transporters

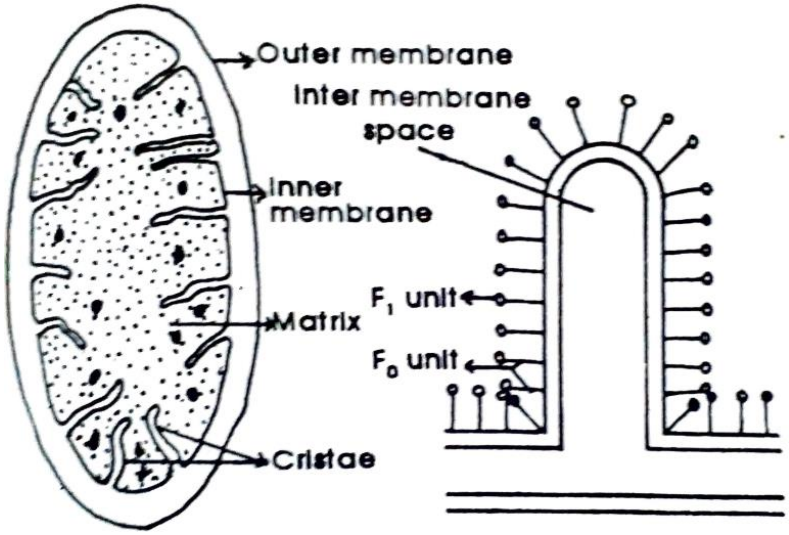

Fig: 5.5. Mitochondria

The respiratory break down of glucose in presence of oxygen is an oxidative process. During aerobic respiration simple carbohydrates and intermediates like phosphoglyceraldehyde, pyruvic acid, isocitric acid, α-ketoglutaric acid, succinic acid and malic acid are oxidized. Each oxidative step involves release of a pair of hydrogen atoms (2H) which dissociate into two *protons* ($2H^+$) and two *electrons* ($2e^-$).

$$2H \rightarrow 2H^+ + 2e^-$$

The pairs of hydrogen atoms ($2H^+ + 2e^-$) released in each oxidative step of Kreb's cycle do not combine directly with oxygen but pass through a series of coenzymes and cytochromes, which form electron transport system, before reacting with O_2 to form H_2O.

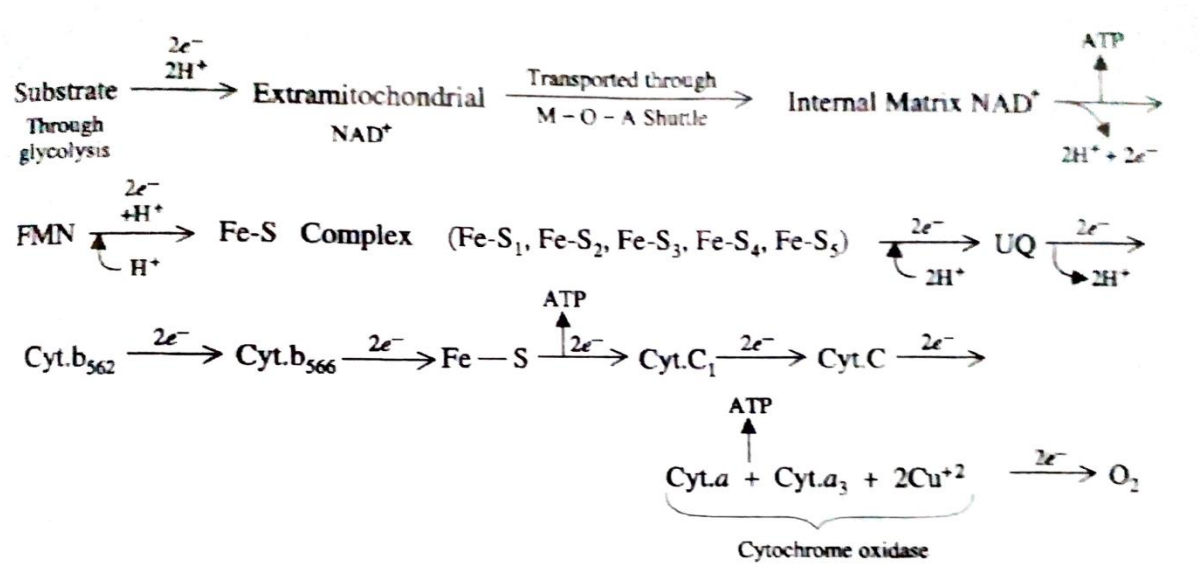

Fig: 5.6 complete set of electron carriers in plants aerobic respiration showing the transfer of electrons and protons both or only electrons as in case of cytochromes.

The electron transport system is made up of following coenzymes and proteins:

1. Nicotinamide adenine dinucleotide (NAD).
2. Flavo proteins (FMN) or FAD
3. Fe-S protein complex
4. Coenzyme-Q (Co. Q) or ubiquinone (UQ)
5. Cytochrome *b* (Cyt. *b)-Cyt* b_{562} *and Cyt* b_{566}
6. Fe-S, protein
7. Cytochrome C1 (Cyt. C1)
8. Cytochrome C (Cyt. C)
9. Cytochrome a (Cyt. a)
10. Cytochrome a3 (Cyt. a_3)

All the above coenzymes are found in F1 particles of mitochondria.

During the transfer of hydrogen atoms from one coenzyme to another coenzyme, a large amount of energy is released which is picked up by ADP to form ATP with the help of inorganic phosphate (iP).

During respiration, the electron pairs liberated rom respiratory compounds are picked up by coenzymes like NAD^+ or $NADP^+$ and FMN etc. The transfer of electrons in all compounds except succinic acid takes place first in NAD^+ or $NADP^+$ and later on in FMN. The transfer of electrons from succinic acid takes place directly to the FAD and not through NAD^+ or $NADP^+$. Due to this reason only two molecules of ATP are produced in the formation of fumaric acid from succinic acid whereas in case of other compounds three ATP molecules are produced because in these cases the electrons are first picked up by NAD.

The different steps of electron transport system are as follows:

1. The hydrogen pairs released from different substrates of Kreb's cycle except succinic acid react with metric NAD^+. Two electrons and one proton (H^+) are transferred and NAD+ causing its reduction and one proton is released in the medium.

$$\underset{}{2H \rightarrow} \underset{\text{(protons)}}{2H^+} + \underset{\text{(electrons)}}{2e^-}$$

2. Now, two electrons and one proton are transferred from NADH to flavoprotein-flavin mononucleotide (FMN) causing oxidation of NADH to NAD^+ and reduction of FMN into $FMNH_2$. One hydrogen ion (H+) is picked up from hydrogen ion pool to complete this reaction.

$$NAD^+ + 2H^+ + 2e^- \rightarrow \underset{\text{(reduced)}}{NADH} + \underset{\text{(ion pool)}}{H^+}$$

   The free energy released at these steps stored during oxidative phosphorylation and one molecule of ATP is synthesized from ADP and inorganic phosphate.

   The hydrogen pair from succinic acid is first transferred to FAD to form $FADH_2$. The $FADH_2$ transfers electrons to Ubiquinon (UQ) through Fe-S and from UQ the electrons pass to cytochromes arranged in normal series on the basis of redox potentials. Thus, during oxidation of succinic acid only 2 ATP molecues are generated.
3. The oxidation of $FMNH_2$ takes place by transferring electrons to Fe-S protein to form reduced Fe-S and oxidized FMN. The protons ($2H^+$) are released in the space.
4. The reduced Fe-S then transfers its 2 electrons to ubiquinone (UQ) or CoQ one by one. The two protons ($2H^+$) are picked up from the matrix (medium). The UQ is reduced to UQH_2.
5. The reduced ubiquinone (UQH_2) then transfers a pair of electrons (2e-) (one at a time) to cytochrome b (Cyt. b) while two hydrogen ions are released in the medium. Thus, UQH_2 is oxidized and Cyt *b is reduced* ($Fe^{+3} \rightarrow Fe^{+2}$).
6. The reduced Cyt. *b* transfers its electron ($2e^-$) to Fe-S protein causing oxidation of Cyt. *b* ($Fe^{2+} \rightarrow Fe^{3+}$) and reduction of Fe-S.
7. The reduced Fe-S protein transfers electrons to Cyt C_1 to reduce it. The energy released at this step is coupled to from ATP from ADP and iP.
8. Reduced Cyt. c_1 transfers its electrons to Cyt. *c* causing reduction of Cyt.*c* and oxidation of Cyt.c_1.
9. Reduced Cyt.*c* transfers a pair of electrons to Cyt. *a causing reduction of Cyt.*a.
10. Pair of electrons are then transferred from reduced Cyt. *a* to Cyt. a_3. Thus, Cyt, a_3 is reduced. The energy released at this step is coupled to form ATP and ADP and iP.
11. Reduced Cyt. *a3* loses a pair of electrons which are accepted by molecular oxygen along with a pair of protons ($2H^+$) from the medium (hydrogen ion pool) to form one molecule of water.

It should be noted that four electrons and four protons (two pairs of hydrogen) will be needed during the real reduction of one oxygen molecule to form water.

$$O_2+4e^-\rightarrow 2(O^-)$$

$$2(O^-) + 4H^+\rightarrow 2H_2O$$

The reduction of various cytochromes requires only electrons and no protons. Each cytochrome possesses an iron element in the centre which functions for accepting $(Fe^{3+} \xrightarrow{+e^-} Fe^{2+})$ or donating $(Fe^{2+} \xrightarrow{-e^-} Fe^{3+})$ electrons. When a cytochrome accepts electrons, it is reduced and if a cytochrome donates electrons, it is oxidized.

Flavin mononucleotide (FMN) is a metalloprtoein and Fe-S protein is iron –sulphur protein. The last step (1) is called *terminal oxidation* which is catalysed by enzyme cytochrome oxidase. This enzyme contains inseparable Cyt. *a* and Cyt.a_3 components and a polypeptide containing two copper ions ($2Cu^{2+}$). Both iron and copper undergo reversible changes in their oxidized states $(i.e.,\ Fe^{2+} \rightleftharpoons Fe^{3+} + e^-;\ Cu^+ \rightleftharpoons Cu^{2+} + e^-)$ during electron transport by cytochrome oxidase.

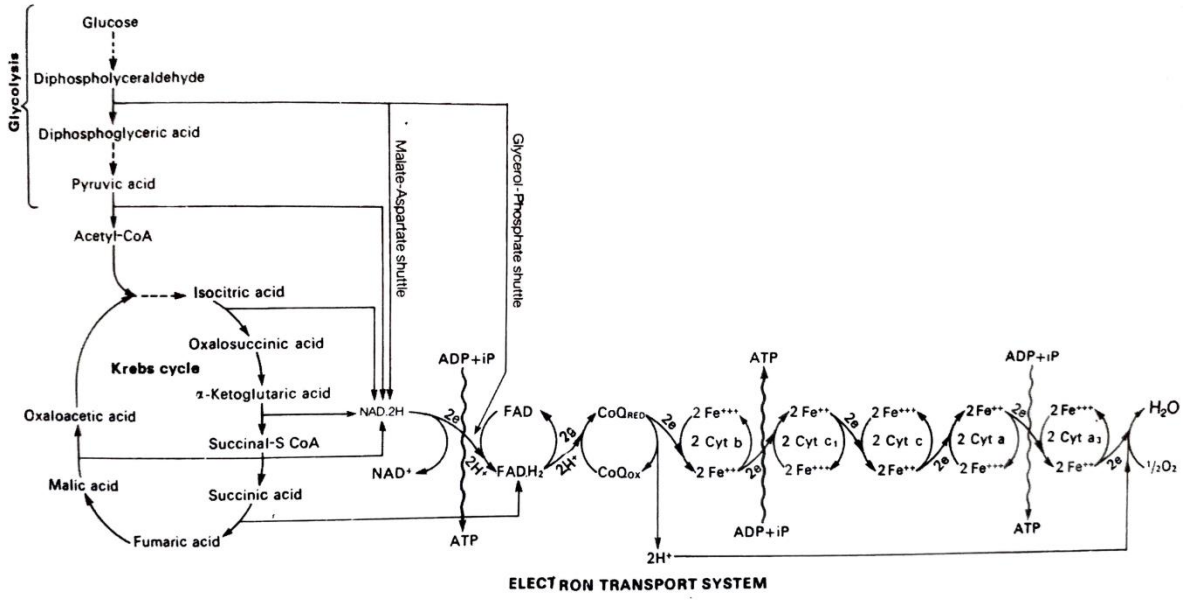

Fig.:5.5. Steps in Electron transport system

Summary of electron Transport system

1. It is made up of coenzymes NAD^+ or $NADP^+$, FAD and coenzyme Q and cytochromes *b*, C_1, C, *a* and a_3.
2. The transfer of electrons in all compounds except succinic acid takes place first in NAD^+ or $NADP^+$ and later on in FAD.
3. The transfer of electrons from succinic acid takes place directly to the FAD.
4. 3ATP molecules are produced for each $FADH^2$ molecules.
5. Only 2ATP molecules are produced for each $FADH^2$ molecule.

6. The reduction of various cytochromes requires only electrons and no protons.
7. The formation of one molecule of water requires ½ O_2+2e^-+2H^+ while reduction of one molecule of oxygen (O_2) requires 4e^- + 4H^+.
8. The reduction and oxidation of coenzymes and cytochromes take place in a sequence and stepwise because in electron transport chain they are arranged in a series according to their redox potential. The firs coenzyme (NAD^+) possesses low redox potential while last cytochrome (Cyt. a_3) highest. Thus, the transfer of electrons proceeds from compounds with low redox potential to those with high redox potential.

Oxidation of Extra-mitochondrial NADH or NADH Shuttle Systems

Normally NADH does not penetrate the inner mitochondrial membrane but it is continuously produced and accumulated in cytosol through glycolytic enzyme 3-phosphoglyceraldehyde dehydrogenase, The NADH produced in the cytosol is called extramitochondrial NADH. This, however, under aerobic conditions does not accumulate and is oxidized by mitochondrial respiratory chain or electron transport chain. It is facilitated through special shuttle systems where the electrons from cytosolic NADH arc carried across the inner mitochondrial membrane by an indirect route. Following two important shuttle systems explaining such penetration of NADH into inner mitochondrial membrane are described here:

Malate-oxaloacetate-aspartate shuttle.

This shuttle is most common and of universal occurrence in plants, in 'heart, liver and kidney of animal mitochondria. It involves three membrane carriers and four enzymes. It is a readily reversible shuttle, *i.e.*, can be operated in both directions either into or out of the mitochondria and its complexity is due to impermeability of mitochondrial membrane to oxaloacetate. When 2NADH are transported through this shuttle, 6ATP molecules will be produced. A net gain of ATP per glucose molecule oxidized will be 38.

This type of shuttle mechanism can be explained through following, steps:

(1) The cytosolic NADH first transfers its electrons to cytosolic oxaloacetate to form malate in presence of enzyme *cytosolic malate dehydrogenase*.

(2) The malate, carrying electrons, easily passes through inner mitochondrial membrane into the matrix by a dicarhoxylate transport system (A).

(3) This malate in matrix transfers its electron to the matrix NAD^+ in presence of *matrix malate dehydrogenase*. This results into reduction of NAD^+ into NADH and formation of oxaloacetate.

(4) The NADH formed in matrix now passes its electrons directly to the respiratory chain (= Electron transport chain) and 3 ATP molecules are generated when this pair of electron passes to O_2.

(5) The oxaloacetate formed in matrix cannot pass back into the cytosol from the matrix through inner membrane. So it is converted into aspartate and a-ketoglutarate by the action of enzyme *transaminase*. The aspartate can pass via the amino-acid transport system (C).

(6) The transport system (B) regenerates oxaloacetate into the cytosol and helps in exchange of aspartate to glutamate.

(7) The α-ketoglutarate is carried out to cytosol through dicarboxylate transport system (A) in exchange of malate which has passed inward.

The net reaction of malate-aspartate shuttle is as follows:

$$NADH + NAD^+ \rightleftharpoons NAD^+ + NADH$$

Cytosolic Mitochondrial Cytosolic Mitochondrial

2. Glycerophosphate-dihydroxyacetone phosphate shuttle or G-3—P-DHAP shuttle. This shuttle is not very common and is found mainly in brain and in the insect rnuscles and other eukaryotic animal cells. It involves membrane carriers and two enzymes: (i) Cytosolic glycerol 3-Phosphate dehydrogenase and (ii) Mitochondrial (matrix) glycerol 3-phosphate dehydrogenase.

It is irreversible or unidirectional shuttle, i.e., it can transfer reducing equivalents only from cytosol to matrix and not vice-versa. When two extramitochondrial NADH (produced during glycolysis) are transported by this shuttle, only 4 ATP molecules will be produced and the net yield of ATP per glucose oxidized will be 36 instead of 38. Glycerol 3-phosphate dehydrogenase is NAD-linked in the cytosol whereas the enzyme found in the matrix is flavoprotein linked.

G-3-P-DHAP shuttle mechanism can be explained through following steps:

(1) The cytosolic NADH first transfers its electrons to cytosolic dihydroxyacetone phosphate (DHAP) in presence of enzyme cytosolic glycerol 3-Phosphate dehydrogenase to form glycerol 3-phosphate (G-3-P).

(2) Glycerol-3-phosphate now enters into the matrix through membrane carrier where it is reoxidized to dihydroxyacetone phosphate (DHAP) in presence of FAD-bound matrix glycerol 3-phosphate dehydrogenase.

(3) The DHAP formed in the matrix now diffuses out of the mitochondria into the cytosol to complete one turn of the shuttle.

The net reaction of G-3-P, DHAP shuttle is as follows:

$$NADH + H^+ + FAD^+ \longrightarrow NAD^+ + FADH_2$$

Cytosolic Mitochondrial Cytosolic Mitochondrial

3. ATP molecules Produced Per Glucose Molecule Oxidized under Aerobic Conditions (Respiration)

(1) Through Malate-Oxaloacetate-aspartate shuttle

$$\text{Glucose} + 38\ \text{Pi}\ (H_3PO_4) + 38\text{ADP} + 6O_2 \longrightarrow 6CO2 + 38\text{ATP} + 44H_2O$$

(2) Through glycerophosphate shuttle

$$\text{Glucose} + 36\ \text{Pi}\ (H_3PO_4) + 36\text{ADP} + 6O2 \longrightarrow 6CO_2 + 36\text{ATP} + 42\ H_2O$$

5.6 ATP SYNTHESIS

Oxidative Phosphorylation

Synthesis of ATP during oxidation of coenzymes in electron transport system of aerobic respiration is called *oxidative phosphorylation.* In this process, *the* substrate is first oxidized by releasing a pair of hydrogen atoms which dissociate into protons ($2H^+$) and electrons $2e^-$. They are picked up by NAD^+ due to which NAD^+ is reduced to NADH + H^+. Reduced NAD (NADH) is oxidized to NAD^+ by transferring one proton and two electrons to FMN. Thus, FMN is reduced to $FMNH_2$ which needs one more proton from the medium. $FMNH_2$ transfers two electrons to ubiquinone (UQ) through Fe-S protein causing reduction of UQ to UQH_2. The UQH_2 transfers its electrons to cytochromes *b* and protons (2H+) to the medium. Thus UQH_2 is oxidized to UQ and Cyt *b* is reduced. The reduced Cyt *b* transfers electrons to Cyt c_1 through Fe-S protein. The protons after reduction of UQ now do not participate in the oxidation and reduction process and only electrons participate in the oxidation and reduction of Cytochromes like Cyt *b,* Cyt. C_1, Cyt. C, Cyt. *a* and Cyt. a_3. In the last step, two protons from hydrogen pool, two electrons released from Cyt. a_3 and half molecule of oxygen (1/2 O_2) combine to form one molecule of H_2O. During this complete process; energy is released at three different steps which is coupled to form ATP from ADP and inorganic phosphate. The oxidation of NADH or NADPH produces three molecules of ATP while that of $FADH_2$ and succinic acid produces only two ATP molecules each. During the formation of succinic acid from succinyl CoA, only one ATP molecule is synthesized and GDP is converted into GTP in presence of inorganic phosphate. This step is an example of **substrate phosphorylation.** The complete oxidation of one molecule of glucose in aerobic conditions produces 38 ATP molecules.

Site of Oxidative Phosphorylation

Mitochondria have been considered the site of oxidative phosphorylation as they contain **coenzymes** of respiratory chain arranged in cristae, ATP synthetase molecules, the enzymes of citric acid cycle (Kreb's cycle) and enzymes of fatty acid oxidation. Each mitochondrion

possesses two membranes; outer and inner, central matrix and an intermembrane space between outer and inner membranes. The inner membrane forms numerous cristae towards central matrix.

The outer membrane contains a few enzymes and is permeable to many small molecules and ions. The inner membrane is impermeable to all ions and uncharged molecules and possesses ET chain, succinate dehydrogenase and ATP synthesizing enzymes. The number of ET chains and enzymes in a single mitochondrion vary from its plain surface to cristae. The intermembrane space contains enzymes *adenylate kinase* and a few other enzymes. The matrix contains most of the enzymes of citric acid cycle, fatty acid oxidation and pyruvate dehydrogenase ystem. Coenzymes ATP, ADP, AMP, NAD, NA DP, CoA, Pi and several ions like K+, Mg^{2+} and Ca^{2+} etc., are also found in the matrix.

ATP synthetase (=F_o F_1 ATPase)

ATP synthetase or F_o F_1 AT Pase is a ATP synthesizing enzyme complex which is found in the inner membrane of mitochondria. It is made up of two components (factors) F_0 and F_1. F_1 component is a knob like structure protruding from the inner membrane and is present towards the matrix while F_0 component is a rectangular piece like structure found embedded in the inner membrane. F_1 and F_0 components remain connected with the help of a stalk.

The F_1 component was isolated and purified from the inner membrane of mitochondria for the first time by *Efraim Racker* and Colleagues (1960). It was observed that when the inner mitochondrial membrane was given the sonic treatment (sonication), the cristae membranes were fragmented which after rejoining and sealing through their ends produced *submitochondrial particles.* However, in such particles the F_1 ATPase components were present outside rather than the inside. Thus, they represented inverted particles. When these inverted *submitochondrial particles* were treated with trypsin or urea, the F_1 spheres or components became detached and could not synthesize ATP from ADP and Pi alone but they could hydrolyse ATP into ADP and Pi. For this reason, they were called *F_1 ATPase.* It was again observed that the remaining F_0 particles can transfer electrons through ET chain present in them but cannot synthesize ATP. When the F_1 detached component (particle with only F_0 component) was mixed with free F_1 spheres, the formation of original sub-mitochondrial particles with F_1 and F_0 both the component took place. They were capable of synthesizing ATP. This suggested that the role of F_1 component is to synthesize ATP.

Mechanism of Oxidative Phosphorylation

Three important theories have been proposed to explain the mechanism of oxidative phosphorylation. These theories explain how the energy transfer between electron transport and ATP synthesis takes place.

(1) Chemical Coupling Hypothesis

It was first proposed by ***Slater* in 1953** and is based on the **principles of** substrate level **phosphory**lation. The hypothesis postulates that a high energy intermediate **is produced** as passed from one carrier to the next. However, no such high energy intermediates have been shown to exist and the need for intact mitochondrial membranes for effective oxidative phosphorylation is not explained by this **hypothesis.**

For **the explanation of** hypothesis it is proposed that two hypothetical coupling factors (enzymes), called **X and E,** are involved at each ATP generating step. It is further proposed that coupling factors required at three ATP generating steps are different. They are designated at X_1 and E_1, X_2 and E_2 **and** X_3 and E_3. Various steps of mechanisms are as follows:

1. **The coupling** factor X first combines with the respiratory enzyme like Cyt. ***b*** to form a high energy intermediate complex ($Fe^{3+} \sim X$)

   $$\mathbf{Fe^{2+} + X} \longrightarrow Fe^{3+} \sim X$$

2. The high *energy* intermediate complex combines with PO^{-}_{4} to form phosphorylated intermediate (X P) containing a high energy phosphate group. At this step the respiratory *enzyme* is removed.

   $$Fe^{3+} \sim X + PO^{-}_{4} \longrightarrow X \sim P + \mathbf{Fe^{2+}}$$

3. The phosphorylated intermediate (X ~ P*)* now combines with another coupling factor *E* to replace first coupling factor *X* and to form energy rich phosphorylated complex (*E* P) which catalyses the synthesis of ATP from ADP and regeneration of the coupling factor (enzyme) *E.*

$$X \sim P + E \longrightarrow E \sim P + X$$

$$E \sim P + ADP \longrightarrow ATP + E$$

Thus, it is presumed that the function of coupling factors X and E is to transfer the energy released in redox reaction for ATP synthesis.

(2) Conformational coupling hypothesis

It was first proposed by *Boyer* in 1964. According to this hypothesis the energy produced during electron transfer is conserved by conformational changes in the molecules comprising the mitochondrial membrane (protein component of respiratory electron carriers) and matrix which may be driving force for ATP synthesis.

Boyer (1965) proposed that there is a direct communication between electron transfer catalysts and ATP synthesizing components through polypeptide polypeptide interaction.

Boyer and Slater (1974) proposed a *modified conformational coupling hypothesis* which postulates that electron transfer induces conformational changes leading to translocation of protons. Conformational changes in electron transfer proteins induce changes in ATP synthesizing protein components. They believe that passage of protons through F_1 can

change the conformation of its protein and such proton induced conformational changes near the active site can synthesize ATP.

ADP and inorganic phosphate can combine spontaneously to form ATP in the active site of F_1 of ATPase without requiring free energy. ATP formed is tightly bound to ATPase. The energy is, however, required to release tightly bound ATP molecules from ATPase. The protons when bind elsewhere other than the active site, can cause conformational changes in F_1 part of ATPase resulting into release of ATP. The protons are released into the solution on M-side of the membrane.

ADP + Pi $\longrightarrow$ ATP (at the active site of F_1 of ATPase

Proton binding to F_1 of ATPase $\longrightarrow$ conformational changes in F_1 of ATPase $\longrightarrow$ Release of ATP

(3) The Chemiosmotic hypothesis

It was proposed by *Peter Mitchell*, a British biochemist, in 1961. This theory is most convincing and acceptable to date. The hypothesis can be explained through following points:

1. The inner mitochondrial membrane possesses three kinds of flow:
   (a) Electron transport, *(b)* Proton translocation *(c)* ATP synthesis.
2. The electron transport system (ETS) is found within inner mitochondrial membrane and the phosphorylating system is found in the head piece (F_0) of ATPase particle.
3. The ATP synthesis and electron transport are coupled through proton translocation or gradient
4. Certain stages of electron transport system involve liberation of hydrogen ions (protons)
   e.g., Malate + NAD^+ $\longrightarrow$ Oxaloacetate + NADH + H^+
   $CoQH_2$ + 2 Cyt *b* (Fe^{3+}) $\longrightarrow$ CoQ + 2 Cyt *b* (Fe^{2+}) + $2H^+$
5. Other *stages* of electron transport system involve uptake of hydrogen ions *e.g.*,
   NADH + H^+ + FAD $\longrightarrow$ NAD^+ + $FADH_+$
   2 Cyt a_3 (Fe^{2+}) + $2H^+$ + ½ O_2 $\longrightarrow$ 2 Cyt a_3 (Fe^{3+}) + H_2O
6. Hydrogen ion uptake reactions take place towards the inner or matrix (M-side) of inner mitochondrial membrane while hydrogen ion liberation or releasing reactions occur outside or cytosol side (C-side) of inner mitochondrial membrane.
7. The electron transfer carriers in the membrane of mitochondrion are arranged in such a way that the transfer of electrons takes place from the carriers of low redox potential to the carriers of high redox potential and couple with transport of protons across the membrane. In other words, electron transport system operates a proton pump which transports protons (H^+) only from M-side to C-side of mitochondrial membrane at high electro-chemical potential or proton motive force (pmf). This movement of protons is called proton translocation. Six protons are generated per electron pair transported.
8. The transport of protons to the C-side of membrane causes positive charges on the outer surface of membrane creating a *proton concentration gradient* or membrane potential across the inner mitochondrial membrane.

9. The proton concentration gradient forces the protons from C-side to M-side. The proton gradient across the membrane generates sufficient electrochemical energy which helps in driving the process of ATP synthesis (oxidative phosphorylation).
10. It is proposed that inner membrane of mitochondria is impermeable to hydrogen ions (H^+) and also to K+, OH^- and Cl^- ions. Due to this reason, the protons do not flow back *i.e.*, from C-side to M-side, directly through the membrane but they flow back into the matrix through a specific region called *proton channel* or *pore* present in the F_0 portion of F_0 — F_1 ATPase (ATP synthetase) molecule. F_1 portion acts as an active site of ATP synthesis.
11. The back flow of protons through proton channel helps in the synthesis of ATP from ADP and Pi. One ATP molecule is produced for every two protons passing through F_0 — F_1 complex.
12. When hydrogen ion move to C-side, the H_2O dissociates into OH^- and H^+ to form water. Thus OH^- efflux is essentially similar to H^+ efflux.

Adenosine Triphosphate (ATP)

ATP is an energy rich compound which acts as a link between cellular exergonic (energy releasing) and endergonic (energy requiring) reactions. It is a triphosphate ester compound of adenine ribonucleoside which is formed by the union of one molecule of purine base - adenine, one molecule of pentose sugar—ribose and three molecules of phosphoric acid. Adenine and ribose combine to form a nucleoside called adenine ribonucleoside. Three phosphate esters of adenine ribonucleoside are found which are called adenosine monophosphate (AMP), adenosine diphosphate (ADP) and adenosine triphosphate (ATP). In all these three compounds (ATP, ADP and AMP) the CH2OH group of ribose forms an ester link with the phosphate group of phosphoric acid, H_3PO_4.

ATP is hydrolysed in presence of suitable enzyme, or by dilute mineral acids, or alkalies. As a result of hydrolysis the terminal phosphate group is released, leaving ADP. The release of standard free energy for this reaction is about —7,600 calories. The hydrolysis of second phosphate group in ADP results in AMP and release of about-6,500 calories energy takes place. The hydrolysis of AMP results in the formation of adenosine and H_3PO_4 and releases only small amount, about-2,200 calories, of energy.

5.7 ENERGY TRANSFER AND CONSERVATION

Within plant cells, most energy is transferred through a carrier—adenosine triphosphate, or ATP, known as the universal currency for energy transfer. Whether helping to convert light energy respiration, ATP acts as an agent to carry and transfer energy into chemical energy during photosynthesis or breaking down glucose in glycolysis and aerobic.

ATP is a nucleotide composed of the nitrogen containing base adenine, the sugar ribose, and three phosphate groups. Energy released through glucose breakdown is used to drive the synthesis of ATP from adenosine diphosphate, or ADP, and inorganic phosphate (P_i):

$$ADP + P_i + energy \rightarrow ATP$$

The energy is largely stored in the bonds linking the phosphate groups. In reactions or processes where energy is needed, ATP releases energy through the hydrolysis and hence the removal of phosphate group:

$ATP + H_20 \rightarrow ADP + P_i$ + energy (7.3 kilocalories per mole)

Sometimes the second phosphate group may also be removed via hydrolysis to generate the same amount of energy and adenosine monophosphate (AMP):

$ADP + H_20 \rightarrow AMP + P_i$ + energy (7.3 kilocalories per mole)

The terminal phosphate group of ATP is not simply removed inmost cases but is transferred to another molecule within a plant cell. This addition of a phosphate group to a molecule is defined as phosphorylation. The enzymes that catalyze such transfers are named kinases.

The following two examples of energy transfer involve ATP. The first is synthesis of sucrose by sugarcane:

$$Glucose + fructose + 2\ ATP + 2\ H_20 \rightarrow sucrose + H_20 + 2\ ADP + 2\ P_i$$

The second example is the complete breakdown of glucose during cellular respiration:

$$Glucose + 6\ O_2 + 36\text{-}38\ ADP + 36\text{-}38\ P_i \rightarrow 6\ C0_2 + 6\ H_20 + 36\text{-}38\ ATP$$

Either ADP or ATP can be recycled through endergonic or exergonic reactions intertwined in the metabolic pathways. In the plant kingdom, energy flow begins with photosynthesis, through which ATP and then high-energy bonds are formed as sugar by the conversion of light energy from the sun.

In respiration, these bonds are broken down to carbon dioxide and water, and energy is released. Some of this energy is used to power cellular processes, but some energy is lost in each energy-conversion step. The energy flow among all other organisms also starts from photosynthesis or plants, either directly or indirectly.

Respiratory Quotient

The ratio of volumes of CO_2 liberated and O_2 used during respiration is called respiratory quotient. It is denoted by R.Q.

$$\text{Respiratory Quotient (R. Q.)} = \frac{\text{volume of } CO_2 \text{ liberated}}{\text{volume of } O_2 \text{ used}}$$

It can be find out through respiratory quotient that which food material is being oxidized during respiration because the different food materials like carbohydrates, fats, proteins and organic acids etc. possess different R.Q. values. The value of R.Q. may be unity or one, less than one or more than one depending upon the substrate used. The R.Q. values in different respiratory substrates will be as follows:

(1) Carbohydrates: When the respiratory substrate is a carbohydrate or hexose sugars, the R.Q. value will be one or unity because one molecule of CO_2 is produced for each molecule of O_2. In other words, the volume of CO_2 liberated will be equal to the volume of O_2 used. It can be shown by the following equation.

$$\underset{\text{(glucose)}}{C_6H_{12}O_6} + 6O_2 \longrightarrow 6CO_2 + 6H_2O + 673 \text{ k.cal. Energy}$$

$$\text{Respiratory Quotient (R. Q.)} = \frac{\text{volume of } CO_2 \text{evolved}}{\text{volume of } O_2 \text{ used}} = \frac{6}{6} = 1$$

In germinated seeds like wheat, oat, barley or paddy etc., the value of R.Q. is always one because in them the respiratory substrate is a carbohydrate.

(2) Fats: If the respiratory substrate is a fat as in case of germinated seeds of mustard, castor, linseed etc., the R.Q. of respiring cells will be less than one because the volume of CO_2 liberated is quite less in comparison to volume of O_2 consumed. The fats always require more amount of O_2 for their oxidation. The oxidation of fat can be shown by taking an example of tripalmitin fat. It takes place usually at the time of seed germination.

$$\underset{\text{(Tripalmitin)}}{2C_{51}H_{98}O_6} + 145O_2 \longrightarrow 102CO_2 + 98H_2O$$

$$\text{Respiratory Quotient (R. Q.)} = \frac{\text{volume of } CO_2 \text{ evolved}}{\text{volume of } O_2 \text{ used}} = \frac{102}{145} = 0.7 \text{ (less than 1)}$$

(3) Organic acids: If the respiratory substrate is organic acid, the R.Q. of respiring cells will be more than one because the volume of CO_2 liberated is more than the volume of O_2 consumed. The acids already contain more O_2, so they further need only a small amount of O_2. The oxidation of malic acid can be taken as an example for this purpose.

$$C_4H_6O_5 + 3O_2 \longrightarrow 4CO_2 + 3H_2O$$
(Malic acid)

$$\text{Respiratory Quotient (R. Q.)} = \frac{\text{volume of } CO_2 \text{ evolved}}{\text{volume of } O_2 \text{ used}} = \frac{4}{3} = 1.33 \text{ (more than 1)}$$

(4) Succulents: The R.Q. value varies under different conditions in succulents like *Opuntia* and *Bryophyllum*.

(a) Dark fixation or Acidification: lt occurs in dark particularly at night when the stomata remain open in succulents. The carbohydrates are incompletely oxidized to organic acids. The incomplete oxidation of glucose molecule results in the formation and storage of malic acid. The CO_2 is also evolved but in very small amount which is again taken back for dark fixation. Thus, ultimately there will be no production of CO_2.

$$2C_6H_{12}O_6 + 6O_2 \longrightarrow 2C_4H_6O_5 + 4CO_2 + 6H_2O$$
(Tripalmitin)

$$\text{Respiratory Quotient (R. Q.)} = \frac{\text{volume of } CO_2 \text{ evolved}}{\text{volume of } O_2 \text{ used}} = \frac{4}{6} = 0.67 \text{ (less than 1)}$$

In dark fixation

$$2C_6H_{12}O_6 + 3O2 \longrightarrow 2C_4H_6O_5 + 3H_2O$$
(Glucose)

$$\text{Respiratory Quotient (R. Q.)} = \frac{\text{volume of } CO_2 \text{ evolved}}{\text{volume of } O_2 \text{ used}} = \frac{0}{3} = 0$$

(b) Deacidification: It takes place in succulents in either light (day time) or prolong darkness. Here, the organic acids act as respiratory substrate. Therefore a good amount of CO_2 is given out but due to closure of stomata at day time this gas (CO_2) does not come out. The trapped gas is fixed gas by the photosynthesis process in presence of light. In this condition on CO_2 will come out from the plant and R.Q. will be zero at day time.

(5) Anaerobic respiration: During this process, the R.Q. value will be infinite (∞) because O_2 is not used while 2 molecules of CO2 are evolved.

$$C_6H_{12}O_6 \longrightarrow 2CO_2 + 2C_2H_5OH$$
(Glucose)

$$\text{Respiratory Quotient (R. Q.)} = \frac{\text{volume of } CO_2 \text{ evolved}}{\text{volume of } O_2 \text{ used}} = \frac{2}{0} = \infty$$

(6) R.Q in red coloured organs of the plant: The red colour in various parts of the plants like petals and leaves etc. is due to presence of anthocyanin, the synthesis of which requires O_2. Such parts of the plant also require O_2 for respiration and there is evolution of CO_2.

It means due to anthocyanin synthesis and respiration processes there is need of more amount of O_2 than the CO_2 evolved. Therefore, R.Q. value will be less than one. For example, red petals of flowers, red leaves and other red parts of the plant.

(7) R.Q. in germinating seeds: In germinating seeds, some part of embryo (radical) is exposed to atmosphere and other part remains concealed within the seed coat. Therefore, aerobic respiration runs in exposed part and anaerobic respiration runs in concealed part. In both the types of respiration CO_2 is evolved but only in aerobic respiration O_2 is used. It means germinating seeds use less O_2 than CO_2 evolved. Therefore, R.Q. will be more than one.

The R.Q. values of different substrates can be measured by Ganong's respirometer. It is made up of two big glass tubes connected with a rubber tube. The upper portion of one glass tube is bulb-like in which the material is kept and it is also graduated. The reading of change in volume is directly read out from the scale.

The apparatus: Ganong's respirometer consists of (i) a glass bulb connected with a graduated glass tube and (ii) a labelling glass tube'. Graduated glass tube and labelling glass tube both are vertically placed and fixed on a stand. The lower narrow ends of both the tubes are connected with a rubber tube. The neck of the bulb has a small hole. A similar hole is found in the side wall of the glass stopper which fits in the opening of glass bulb. The holes of stopper and neck remain in a line with one another.

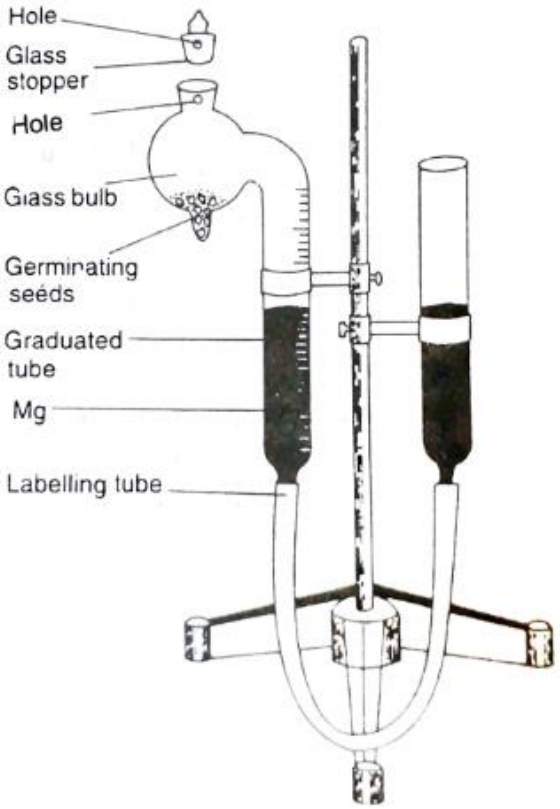

Fig: 5.6. Ganong's respirometer

Method: Fill the graduated and labelling tubes nearly half with mercury or saline water. Pure water should not use because it absorbs CO_2. Now open the mouth of bulb by removing the stopper and place some wet cotton in the narrow bottom of the bulb. Place any respiring plant material (usually germinating seeds containing either carbohydrate or fat or protein) on the wet cotton. Close the stopper in such a way that the hole of the stopper comes in line with the hole of the bulb. The air of the bulb is now in direct communication with outer atmosphere. If the respiring material is green tissue, the bulb must be covered with black paper to check photosynthesis. Adjust the labelling tube by lowering or raising so that the level of mercury or saline water in both the tubes comes to the same level. Now, rotate the stopper to disconnect the internal atmosphere of the bulb with the atmospheric air. Note the initial level of the mercury or saline water in the graduated tube. Keep the apparatus for a few hours and note the second reading.

5.8 SUMMARY

In plant respiration, reduced cellular carbon generated during photosynthesis is oxidized to CO 2 and water, and this oxidation is coupled to the synthesis of ATP. Respiration takes place in three

main stages: glycolysis, the citric acid cycle, and oxidative phosphorylation. The latter comprises the electron transport chain and ATP synthesis.

In glycolysis, carbohydrate is converted in the cytosol to pyruvate, and a small amount of ATP is synthesized via substrate-level phosphorylation. Pyruvate is subsequently oxidized within the mitochondrial matrix through the citric acid cycle, generating a large number of reducing equivalents in the form of NADH and FADH 2.

In the third stage, oxidative phosphorylation, electrons from NADH and FADH 2 pass through the electron transport chain in the inner mitochondrial membrane to reduce oxygen. The chemical energy is conserved in the form of an electrochemical proton gradient, which is created by the coupling of electron flow to proton pumping from the matrix to the intermembrane space. This energy is then converted into chemical energy in the form of ATP by the Fo F_1 -ATP synthase, also located in the inner membrane, which couples ATP synthesis from ADP and Pi to the flow of protons back into the matrix down their electrochemical gradient.

Aerobic respiration in plants has several unique features; including the presence of a cyanide-resistant alternative oxidase and multiple NAD(P)H dehydrogenases, none of which pumps protons. Substrate oxidation during respiration is regulated at control points in glycolysis, the citric acid cycle, and the electron transport chain, but ultimately substrate oxidation is controlled by the level of cellular ADP. Carbohydrates can also be oxidized via the oxidative pentose phosphate pathway, in which the reducing power is produced in the form of NADPH mainly for biosynthetic purposes. Numerous glycolytic and citric acid cycle intermediates also provide the starting material for a multitude of biosynthetic pathways.

More than 50% of the daily photosynthetic yield can be respired by a plant, but many factors can affect the respiration rate observed at the whole-plant level. These factors include the nature and age of the plant tissue, as well as environmental factors such as light, oxygen concentration, temperature, and CO 2 concentration.

5.9 GLOSSARY

Aerobic respiration: respiration with using oxygen.

Anaerobic respiration: respiration without using oxygen.

ATP: Adenosine triphosphate - molecule used to store energy in the body

Carbohydrase: enzyme that breaks down carbohydrates into simple sugars

Carbohydrates: one of the main groups of nutrients; e.g. glucose, starch

Carbon cycle: a natural cycle through which carbon moves by respiration, photosynthesis and combustion, in the form of carbon dioxide

Carbon dioxide: gas produced during cellular respiration.

Cellular respiration: the process where plants and animals convert the energy in sugar to ATP energy.

Electron transport system (ETS): Electron carrier complexes and individual carriers embedded in the inner membrane of mitochondria and in thylakoid membranes of chloroplasts. Responsible for the conservation of energy by electron transport.

Ethanol: product of anaerobic respiration in plant and yeast cells; used in the manufacture of alcoholic drinks

FAD: Flavin adenine dinucleotide. It is a part of complex II and an energy carrier from succinate to complex II of the electron transport system. It is synthesized from the vitamin riboflavin (B2).

Glycolysis: First step of cellular respiration – breakdown of glucose into two molecules of pyruvate (occurs in the cytoplasm).

Krebs cycle (Citric acid cycle): The sequential (and cyclic) oxidation of substrates and conservation of energy by enzymes of the mitochondrial matrix.

Mitochondria: organelle where cellular respiration occurs.

NAD: Nicotinamide adenine dinucleotide (NAD). An energy carrier molecule, used directly by enzymes or to shuttle energy to the electron transport system. Synthesized from the vitamin niacin (nicotinic acid).

5.10 SELF ASSESSMENT QUESTION

5.10.1 Multiple Choice Questions

1. Chief site of respiration in plants is
   a) lysosomes b) mitochondria
   c) ribosomes d) chloroplasts

2. Glycolysis takes place in
   a) Cytoplasm b) chloroplasts
   c) ribosomes d) mitochondria

3. The end product of glycolysis is
a) pyruvic acid b) ethyl alcohal
c) glucose d) carbon dioxide

4. The importance of Kreb's cycle is
a) production of amino acids b) production of vitamins
c) production of ATP molecules through oxidative phosphorylation d) to encourage gluconeogenesis

5. Biological oxidation in Kreb's cycle involves
a) N_2 b) CO_2
c) O_2 d) SO_2

6. The end products of aerobic respiration in plants are
a) CO_2, H_2O and energy b) H_2O and energy
c) CO_2 and energy d) CO_2, and H_2O

7. Common source of energy for cellular activity is
a) ATP b) NAD
c) Fat d) Protein

8. Fructose-1-phosphate is changed to Fructose 1,6 diphosphate by
a) Phosphoglycerate b) Phosphofructokinase
c) Phosphatase d) Enolase

9. Pyruvate dehydrogenase enzyme is used in conversion of
a) glucose to pyruvate b) pyruvic acid to lactic acid
c) pyruvate to acetyl-CoA d) pyruvate to glucose

10. Currency of cell is
a) Granum b) Mitochondria
c) ATP d) Fat

11. The net gain of energy from one gram mole of glucose during aerobic respiration is
a) 38 ATP b) 40 ATP
c) 36 ATP d) 2 ATP

12. Oxidation of $NADH_2$ yields
a) 1 ATP b) 2 ATP
c) 3 ATP d) 4 ATP

13. Complete oxidation of one molecule of acetyl Co-A results in the formation of
a) 12 ATP b) 15 ATP
c) 19 ATP d) 38 ATP

14. The process of Acetyl Co-A synthesis from pyruvic acid is called
a) Reduction b) Dehydrogenation
c) Dephosphorylation d) Oxidative decarboxylation

15. Hydrogen atoms released at succinate level in Kreb's are accepted by
a) FAD b) NAD
c) NADP d) AMP

16. Correct sequence of electron acceptor in ATP synthesis is
a) Cyt. a_1, a_3, b, c b) Cyt. b, c, a, a_3
c) Cyt. c, b, a, a_3 d) Cyt. b, c, a_3, a

17. The reactions of Krebs cycle take place
a) in cytoplasm b) in mitochondria
c) on surface of mitochondria d) in ER and cytoplasm

18. Centre of phosphorylation is
a) Oxysome b) Peroxisome
c) Ribosomes d) Mitochondria

19. Production of ATP by oxidative phosphorylation is driven by energy from
a) Coenzyme A b) $NADH_2$
c) Pyruvic acid d) Movement of protons from inner mitochondrial membrane to cytoplasm

20. RQ fat is
a) More than one b) Zero
c) One d) Less than one

Answers.

1. (b) 2. (a) 3. (a) 4. (c) 5. (c) 6. (a) 7. (a) 8. (b) 9. (c) 10. (c) 11. (a) 12. (c) 13. (a) 14. (d) 15. (a), 16. (b) 17. (b) 18. (a) 19. (b) 20. (d)

5.10.2 Short Answer Questions.

1. Define respiration. What are respiratory substrates?
2. Give types of respiration.
3. Differentiate between aerobic respiration and anaerobic respiration.
4. Why anaerobic respiration produce less energy than aerobic respiration?
5. What is the R.Q.?
6. When will the value of RQ be 1 and when it will be less than 1?
7. Comment upon total gain of ATP in TCA cycle.
8. Write the role of hexokinase in glycolysis.
9. Write the importance of pyruvate dehydrogenase in respiration.
10. Comment upon that TCA is an amphibolic process.

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5.13 TERMINAL QUESTIONS

1. What is respiration? Explain its types with overall equations.
2. Distinguish between respiration and combustion.
3. What do you mean by R.Q.? Give the significance of R.Q.
4. What is anaerobic respiration? Why does anaerobic respiration produce less energy than aerobic respiration?
5. What is respiratory substrate? Enumerate the steps involved in the breakdown of carbohydrates into pyruvic acid.
6. Write short notes on: a) Glycolysis b) Electron transport Chain
7. Differentiate between glycolysis and Krebs cycle.
8. Distinguish between respiration and photorespiration.
9. Discuss TCA cycle with special reference to enzymes.
10. What is oxidative decarboxylation? Write different steps involved in conversion of pyruvic acid into acetyl Co A

UNIT-6-NITROGEN FIXATION AND METABOLISM

Contents:

6.1 OBJECTIVES

- To study the process of nitrogen fixation through nitrogen cycle.
- To learn about the difference between asymbiotic and symbiotic nitrogen fixation.
- To assimilate nitrogen in different parts like leaves, roots etc.
- To understand the process of nodule formation in the host plant during bacterial infection.

6.2 INTRODUCTION

The atmosphere is abundant in nitrogen but nitrogen uptake by the plants does not occur by simple difference of concentration gradient between the outer environment and inside the plant cells. Nitrogen is important for the synthesis of essential biomolecules like amino acids and nucleic acids which are responsible for the synthesis of larger molecules like proteins and genetic molecule, comprised of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Nitrogen has other functions too like it comprise the part of cell membrane deciding the integrity of cell, enzymes and many other signaling molecules. It clearly signifies the importance of nitrogen for the continuity of life. The nitrogen uptake is crucial for plants and certain enzymes and transporters are involved in the process.

6.3 NITROGEN CYCLE

The nitrogen cycle starts with the step of nitrogen reduction in which atmospheric nitrogen is fixed by micro-organisms and bacteria into ammonia (NH_3 or NH_4^+) and this step is also referred to as nitrogen fixation. Ammonia can be used by many micro-organisms and it can also be converted into nitrite (NO_2^-) and then nitrate (NO_3^-) through the energy driven oxidation. The ammonia which reaches the soil is all converted into active nitrate and this process is known as nitrification. The enzymes nitrite reductases and nitrate reductases help plants to take up reduced nitrite and nitrate into ammonia by plants and many other micro-organisms. The nitrogen is further fixed in biomolecules like amino acids from ammonia and these amino acids enter the soil when plants die and degraded by micro-organisms, ammonia re-enter the soil as it was once taken up by plants from soil only. Also, animals consume plants for various essential and non-essential amino acids to build proteins. The nitrifying bacteria convert ammonia into nitrite and nitrate. The fixed nitrogen (nitrite and nitrate) is converted to atmospheric nitrogen by bacteria through the process of denitrification under anaerobic conditions is a way to replenish nitrogen back to atmosphere to maintain balance. Another class of bacteria called anammox which promote anaerobic ammonia oxidation for conversion ammonia and nitrite to N_2 (Figure 6.1).

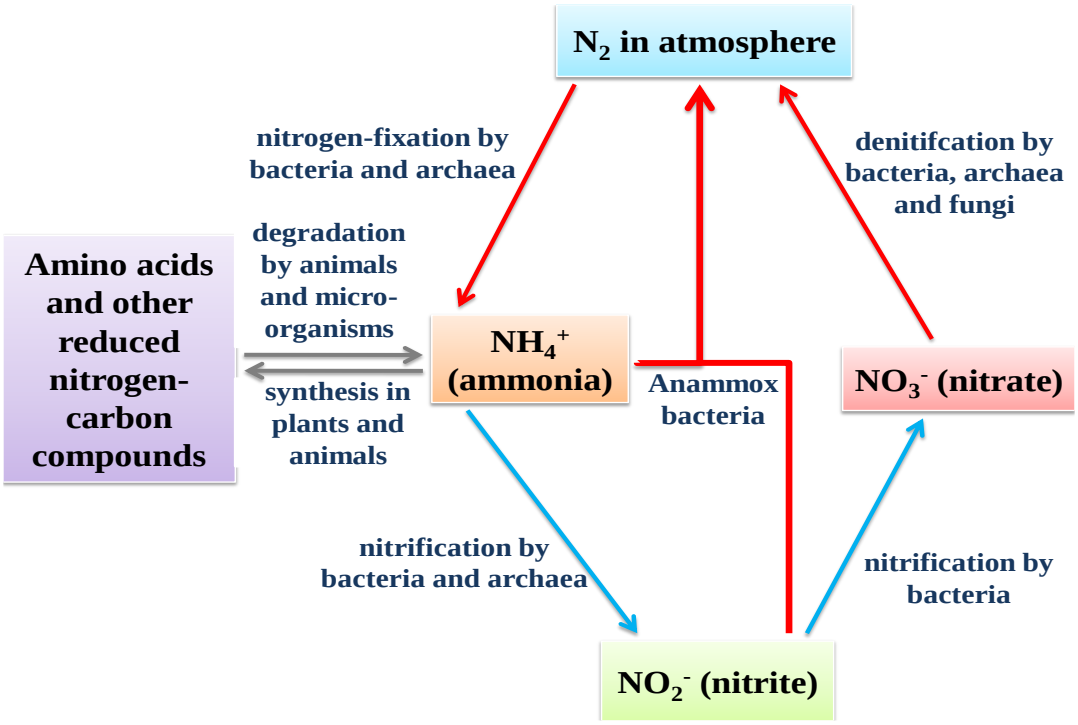

Figure 6.1: Anaerobic ammonia oxidation for conversion ammonia and nitrite to N_2

6.4 ASYMBIOTIC AND SYMBIOTIC NITROGEN FIXATION

The nitrogen fixation can occur with free-living bacteria as well as the bacteria which live in symbiosis with host plant through the formation of nodule and process is called asymbiotic nitrogen fixation or non-symbiotic nitrogen fixation whereas the nitrogen fixation which involves the symbiotic relationship with the bacteria and the host plant is known as symbiotic nitrogen fixation.

The nitrogenase complex catalyzes the process of nitrogen fixation. The complex system includes nitrogenase reductase and nitrogenase as the main components of enzyme complex system. The nitrogenase complex is present in the cytoplasm of bacteroids. NADH formed in citrate cycle transfer electrons to soluble ferredoxin which are from ferredoxin transferred to nitrogenase complex. The ferredoxin, a single-electron or one-electron carrier is made up of two identical subunits forming a 4Fe-4S cluster. The conformation of protein changes when ATP binds to 4Fe-4S cluster post nitrogenase reductase reduction. 2 ATP molecules bind with the protein which later on hydrolyzed to release ADP and phosphate molecules which restored the redox potential and the protein is again ready to take up electron from the ferredoxin molecule.

Nitrogenase is a tetramer, made up of two subunits of α and β each, making $\alpha_2\beta_2$ which possess two catalytic centers, a P cluster which contains two 4Fe-S clusters and an iron molybdenum cofactor (FeMoCo). It can be duly noted that studies suspect electrons to be delivered to the Nitrogenase centre, FeMoCo through the P cluster which are essential for

nitrogen fixation (Figure 2). Nitrogenase reduces nitrogen molecule (N_2) releasing per one molecule of hydrogen per N_2 reduced (Figure 6.2).

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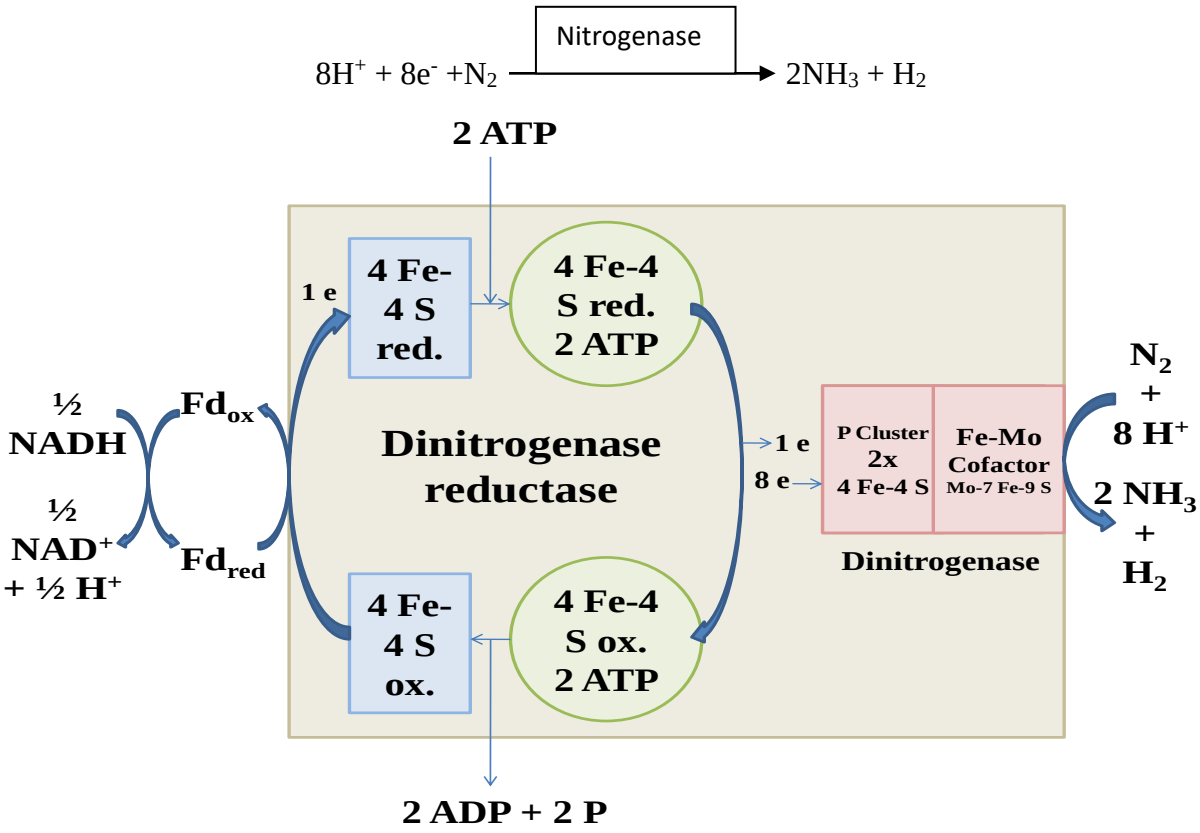

Figure 6.2: The structure of nitrogenase complex and its different constituents which takes part in the transfer of electrons and the process of nitrogen fixation.

The overall nitrogen fixation reaction can be summarized as:

$$N_2 + 4NADH + 4H^+ + 16ATP \longrightarrow 2NH_3 + H_2 + 4NAD^+ + 16ADP + 16P$$

The nitrogenase complex contains highly conserved protein sequences and is made up of two central components which are dinitrogenase reductase and dinitrogenase(Figure 6.3). Six electrons are required for the reduction of one molecule of N_2 and two molecules are needed for the production of H_2, making complete eight electrons required for nitrogen fixation by highly reduced dinitrogenase enzyme. The electrons are transferred from pyruvate molecule reducing ferredoxin.The ferredoxin molecule transfers a single electron molecule to dinitrogenase reductase enzyme. The electrons are transferred from reduced molecule of ferredoxin and when electron is transferred to the dinitrogenase reductase molecule, ferredoxin molecule is oxidized. Electrons are transferred from dinitrogenase reductase to dinitrogenase reducing it in the process. The electrons are transferred from one at a time in which a reduced dinitrogenase reductase

transfers a single electron and after transferring electron to dinitrogenase, dinitrogenase reductase is oxidized. This cycle continues to transfer electron from one carrier to another and it requires hydrolysis of two ATP molecules through the dimeric reductase enzyme.

The hydrolysis of ATP takes place before the actual transfer of one electron to dinitrogenase. The nitrogenase reductase conformation change occurs in two regions due to the ATP binding which are structurally homologous. The conformation change causes the 4Fe-4S centre of reductase enzyme come closer to the P cluster of dinitrogenase. This change helps in facilitating electron transfer from reductase and dinitrogenase (Figure 6.3).

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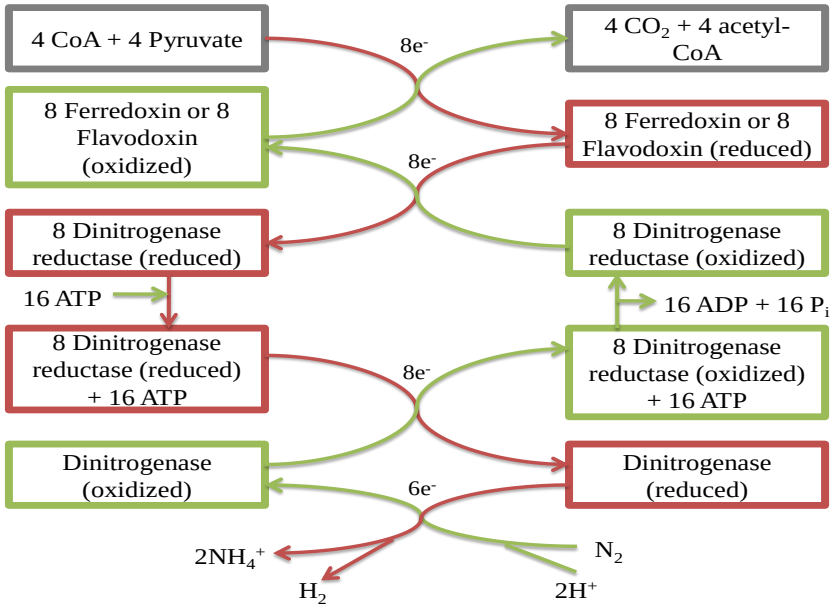

Figure 6.3: The flow of electrons in the process of nitrogen fixation by the nitrogenase complex

It must be stated that nitrogenase enzyme is particularly susceptible to oxygen and it can function properly on low oxygen concentrations. It is due to this reason nodules form an anaerobic i.e. the oxygen-deficient environment and nitrogen fixation takes place in the presence of either limited or very low concentration of oxygen. Bacteroid cytochrome-a/a_3 complex has high affinity towards oxygen and hence the process of respiration can take place in very limited amount of oxygen (~10^{-9} mol/L) too. For nitrogen fixation, a total of 16 molecules of ATP are required and mitochondrial respiratory chain generated 2.5 molecules of ATP per each NADH molecule oxidation. In the case of bacteria, 2 molecules of ATP are produced for the each molecule of NADH oxidation (Figure 6.4). It clearly implies that 16 molecules of ATP are being produced on four molecules of O_2 consumption. If hydrogenase enzyme is present in bacteroid for oxidation of H_2 molecule, a further increase in oxygen consumption is observed. In the nitrogen fixation in bacteroids, five molecules of NADH and one molecule of $FADH_2$ is produced trough the complete oxidation of malate by the citric acid cycle.

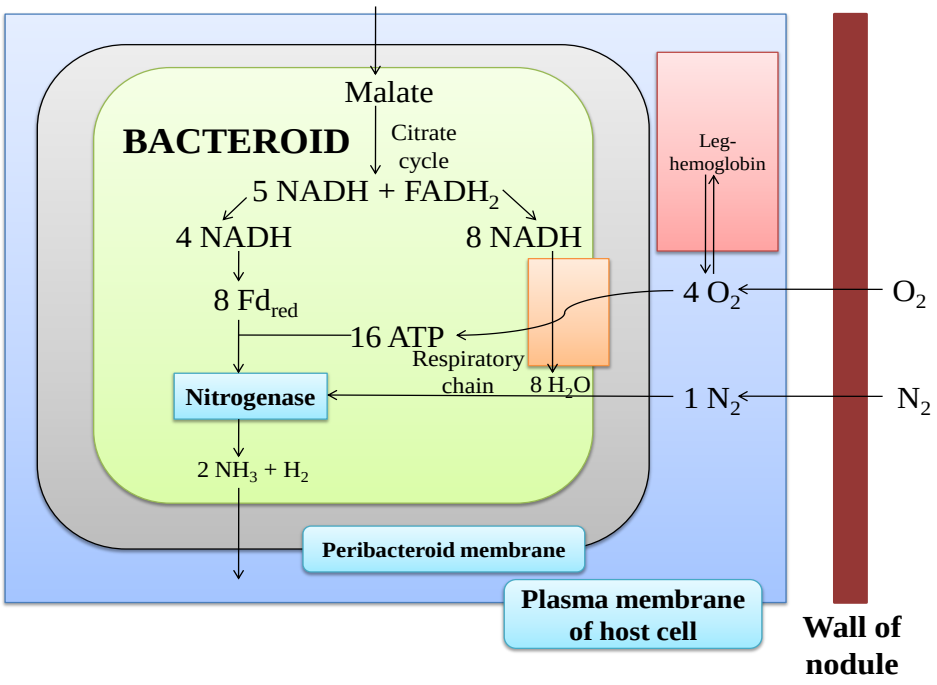

Figure 6.4: The process of nitrogen fixation in bacteroids.

6.5 NITROGEN ASSIMILATION

Nitrate assimilation is a two step process. Enzyme nitrate reductase is present in the cytosol of mesophyll cells whereas nitrite reductase is present in the chloroplasts. Nitrate (NO_3^-) is reduced to nitrite (NO_2^-) by the action of enzyme nitrate reductase, marking the first step of nitrogen assimilation. The enzyme is made up of three domains which are one FAD (flavin adenine dinucleotide) (Domain 1), one heme of the cytochrome-b type (cyt-b_{557}) (Domain 2) and the molybdenum cofactor (MoCo) (Domain 3). The molybdenum cofactor contains a pterine ring with a side chain in the structure to which two sulfur bonds are attached to the molybednum.

The second step involves nitrite (NO_2^-) reduction due to the enzymatic action of nitrite reductase.The enzyme nitrate reductase is a large, soluble protein in which electron flow takes place through cysteine –SH group, FAD, a cytochrome cyt b_{557} and a cofactor contain molybdenum. Total 6 electrons are needed for another enzyme, nitrite reductase. Ittakes up electrons from ferredoxin molecule through light-dependent reaction of photosynthesis by which one electron at a time and total 6 electrons are passed on to the 4S-4Fe center and then to siroheme group which is a heme-like molecule from ferredoxin to nitrite which in is responsible for reduction of NO_2^-into NH_4^+ (Figure 6.5). In the case of non-photosynthetic microbes, the electrons are passed on to the enzyme through the NADPH molecules. The electrons are transported through the photosystem I via photosynthetic electron transport. Nitrite is generally toxic to cells and enzyme, nitrite reductase show high affinity towards nitrite. It is because of this fact; all the nitrite converted by nitrate reductase can be easily converted into ammonia by the action of nitrite reductase in the chloroplast, preventing cell from the toxicity of nitrite.

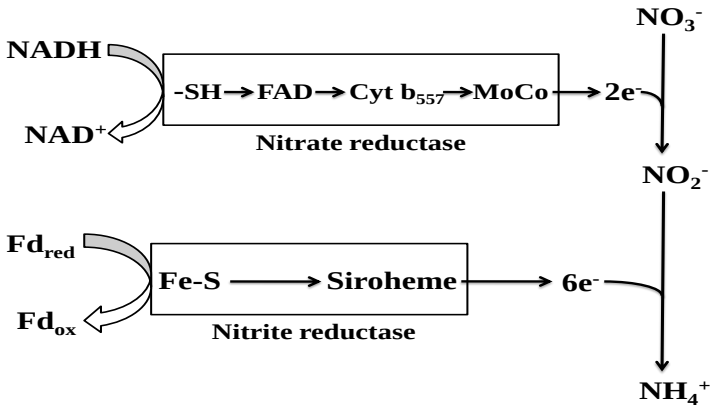

Figure 6.5: The electron transfer in the nitrate reductase and nitrite reductase for the reduction of nitrate (NO_3^-) to nitrite (NO_2^-) and then nitrite to ammonia (NH_4^+).

Nitrate assimilation takes place in roots and leaves majorly. Nitrate assimilation in the roots is essential for the plant growth in early growth and development. The process starts with a symport transport of two protons inside the root cells which lead to the formation of a proton gradient across the plasma membrane by H^+-P-ATPase which is responsible for nitrate uptake within the roots against the concentration gradient. The energy required in the form of ATP is provided by mitochondrial respiration. Root cells can store nitrate in the form of reduced NH_4^+ in the epidermal and cortical cells of the roots. The NH_4^+ is mainly used during the synthesis of amides such as asparagine and glutamine. The amides are transported through xylem vessels into the leaves where it can be stored in the vacuoles in large quantities for a longer period of time and used as per need. It is the process of transpiration which is responsible for nitrate uptake within the different upper tissues of the plants and mesophyll cells take up nitrate possibly through proton symport again (Figure 6.6).

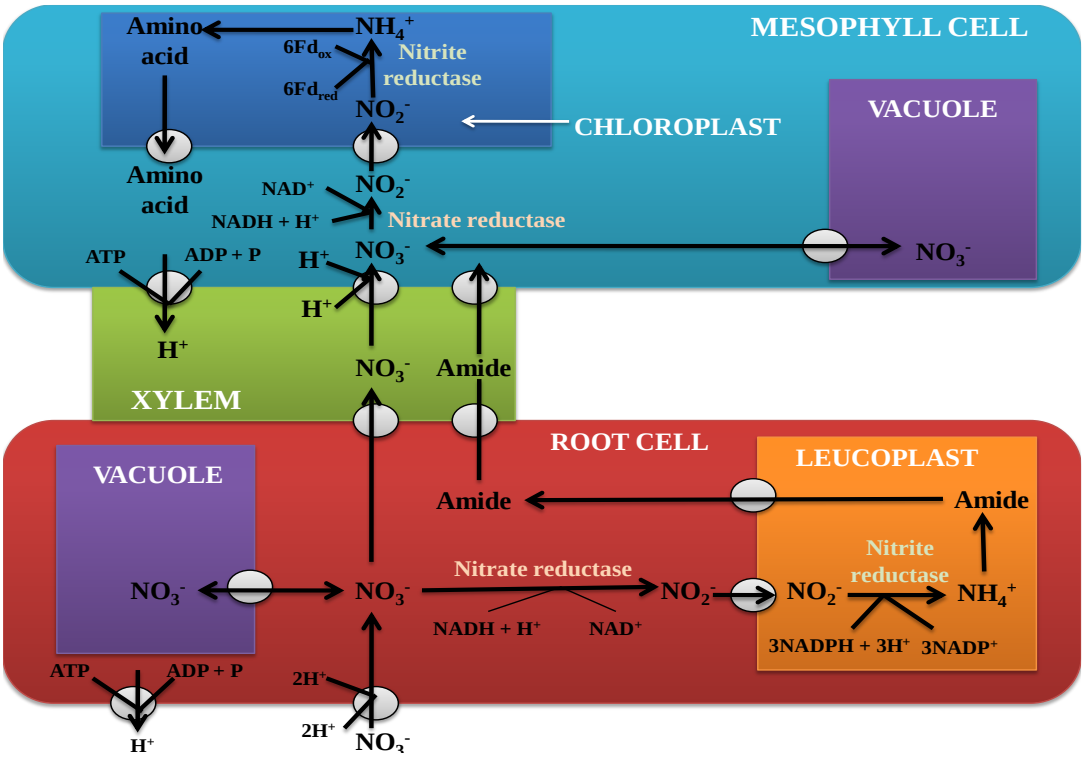

Figure 6.6: The process of nitrate assimilation in the roots and leaves of a plant.

The enzyme glutamine synthetase transfers ammonia (NH_4^+) to the glutamate, forming the glutamine at the expenditure of ATP (Figure 6.7). Glutamine synthetase has high affinity towards ammonia which results all ammonia conversion and taken up which was reduced by nitrite reductase. Small amount of glutamine synthetase is responsible for nitrate assimilation and isozymes of glutamine synthetase are also containing in the cytosol of leaves. Glufosinate acts as an analogue of glutamate and inhibitor for the glutamine synthesis. The ammonia is toxic to living cells and glufosinate inhibit the synthesis of glutamine from ammonia which causes the accumulation of ammonia and cells are killed as a result. Glutamate synthase, which can also be called as glutamine-oxoglutarate amino transferase or GOGAT, convert glutamine into two molecules of glutamate by reacting with α-ketoglutarate in the chloroplast. α-ketoglutarate is transported into the chloroplast through a translocator in which malate is exchanged from the α-ketoglutarate needed for glutamate synthase reaction. There is another translocator which again translocate α-ketoglutarate into the cytosol replacing malate, exporting glutamine outside the chloroplast.Ferredoxin molecule acts as reductant for the reaction. Azaserine act as an inhibitor for glutamate synthase and is also toxic to the plants.

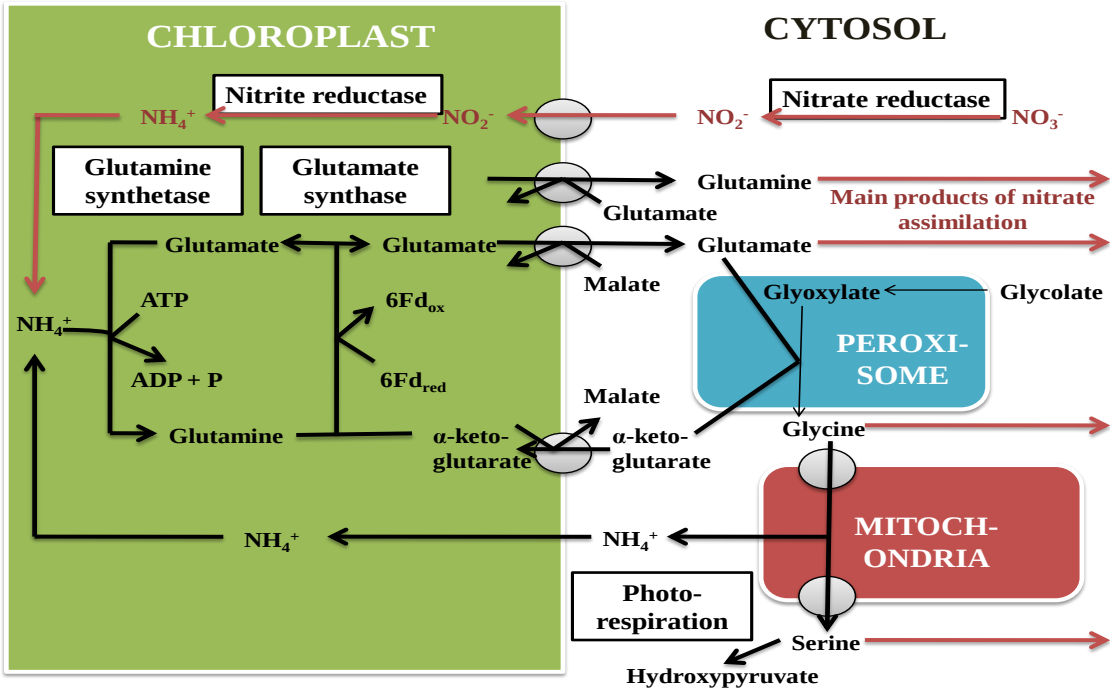

Figure 6.7: The production of main products of nitrate assimilation.

6.6 NITROGEN FIXING PLANTS

Certain bacteria and cyanobacteria can synthesize ammonia from atmospheric nitrogen and they live in symbiosis with some plants to provide them with organic nitrogen. The nodules of legumes were previously thought due to the result of some disease but the process of N_2 fixation and its importance was later explained by H. Hellriegel and H. Wilfarth in 1888. It was revealed that these plants do not need nitrogen fertilizers as a need of supplement as plants with nodules were able to synthesize nitrogen themselves. The most common nodule-inducing bacteria include species from genera, *Rhizobium*, *Bradyrhizobium* and *Azorhizobium* and they are together referred to as rhizobia.Generally, Rhizobia are strictly aerobic gram-negative rods which grow heterotrophically but some species grown autotrophically in the presence of H_2 as *Bradyrhizobium*.Leguminous plants belonging to a large family *Leguminosae* with almost 20,000 species which include soyabean, lentil, pea, clover, lupines, etc. form symbiosis with rhizobia. *Azolla*, the water fern shows symbiotic association with cyanobacteria *Nostoc* to provide nitrogen to rice fields. Woody plants are known to form symbiotic association with N_2 fixing actinomycetes of the genus *Frankia*.

The rhizobium uptake into the host plant is the result of controlled infection which has been still partially understood. The nod factors or nodulation factors are formed lipochito-oligosaccharides that have specific structure characteristics due to acetylation, acylation or sulfation. The receptor kinases from the host bind to the nod factors to transduce a chain signal to

induce curling of root hairs and division of root cortex cells, forming the nodule primordium. The rhizobia then enter the host plant through curled root hairs, forming an infection thread extending into the cortex cells of roots, branch itself to spread infection to nodule primordium, thus developing the nodule. A symbiosome membrane or peribacteroid membrane is formed which encloses the bacteria which enter into the plant through infection thread. The symbiosome allowed the separation of bacteria from the cytoplasm of host cell. After differentiation of rhizobia in the symbiosome, bacteroids are formed, which are surrounded by peri-bacteroid membrane (Figure 6.8).

Many genes in bacteria are responsible for causing infection in host cells but these genes remain inactive when bacteria are free-living. The Nitrogen fixation is important for the production of proteins by these genes which mainly are nif and fix genes. The nod genes are responsible for the induction of nodule formation. Several flavonoids act as signal for plants to formnodules. A constitutive gene, nod gene encodes a bacterial protein which binds with flavonoids. Upon binding of nod gene with flavonoid, other nod genes are activated which are involved in the syntheisi of nod factors as described in above text. Nodulins like leghemoglobin are the proteins which are synthesized by the host plant during infection for the formation of nodules. Nodulin genes are responsible for encoding nodulin proteins.Nodulins are differentiated into early and late nodulins; early nodulins are involved in bacterial infection in the host plant and nodule formation whereas late nodulins are synthesized after the nodule formation.

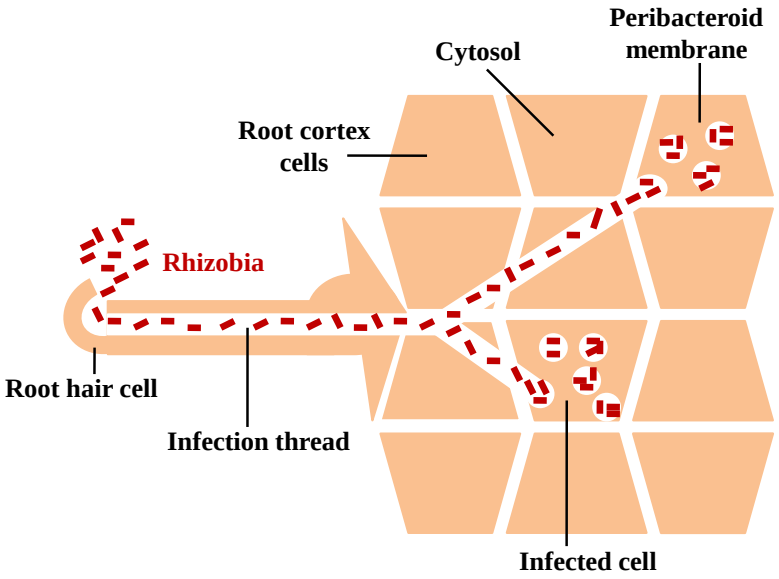

Figure 6.8: Controlled infection by rhizobia to the host plant, formation of infection thread and branching of infection thread to spread the infection from the curly root hairs to root cortex cells.

Vascular tissues connect nodules with roots which help in substrates supply formed by photosynthesis. The host cells provide malate as a main substrate to the bacteroids which is synthesized by the sucrose and delivered by the sieve tubes. The enzyme sucrose synthase is responsible for the metabolizing sucrose. It is degraded to phosphoenolpyruvate in the process of

glycolysis, which later on carboxylation produces oxaloacetate and then at last reduced to malate. Ammonia absorbed by the roots of host plant is converted into asparagine and glutamine which are transported to other parts of plants through xylem vessels (Figure 6.9). The bacteroids oxidize malate by the citric acid cycle.

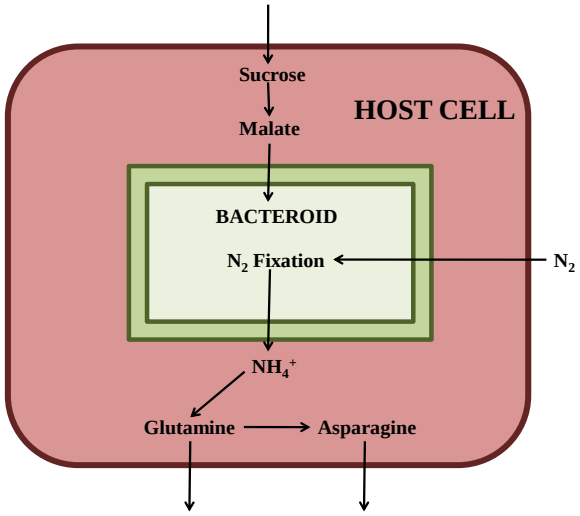

Figure 6.9: Metabolism of different substrates needed by infected host cell.

6.7 SUMMARY

The nitrogen (N_2) contributes to the 80% of earth's atmosphere. It is not available to plants unless it is present in reduced form. The free living bacteria or symbiotic bacteria form rhizobia for fixing nitrogen in the root nodules of leguminous plants. The nitrogenase complex is responsible for the conversion of nitrogen (N_2) to ammonia (NH_3 or NH_4^+) which marks as a step for nitrogen fixation which involves ATP consumption and transfer of 8 electrons to reduce nitrogen. After that nitrogen is assimilated in the roots and leaves of the plants in the form of nitrate (NO_3^-) and nitrite (NO_2^-) by the action of enzyme nitrate reductase and nitrite reductase. Nitrate (NO_3^-) is reduced to nitrite (NO_2^-) by the enzyme nitrate reductase, marking the first step of nitrogen assimilation and another step involves the activity of nitrite reductase in which nitrite (NO_2^-) is reduced to ammonia (NH_3 or NH_4^+). After the assimilation of nitrogen inside the plant cells and tissues, amino acid; glutamine and asparagines synthesis takes place in a defined manner involving certain enzymes like glutamine synthetase and glutamine synthase.

6.8 GLOSSARY

Analog – a compound which has a similar structure to that of another compound.
Biomolecules – these are the substances which are produced by cells and living organisms.
Conformation – it is the distinct arrangement of atoms in any molecule which can readily interconvert.

Constitutive gene – a gene which is expressed all the time.
Fertilizer – a natural or chemical substance use for increasing the fertility of the soil and growth of a plant.
Flavonoids – they are group of plant metabolites with function of health benefits and many others.
Metabolite – it is a substance which is synthesized in the process of metabolism.
Photosynthesis – it is the process of synthesizing oxygen and energy (sugar) using water and carbon dioxide in the presence of sunlight.
Reductase – it is an enzyme which catalyzes the reduction process.
Symbiosis - related to relation between two dissimilar organisms.
Symport –the transportation of two molecules in the same direction
Synthetase –an enzyme which causes the ligation of two molecules at the expense of energy molecules like ATP.
Synthase –an enzyme which causes the ligation of two molecules without spending any energy molecules like ATP.
Substrates–these are the substances with which enzymes react.
Transducer – to convert a substance into another.
Translocators – the molecule which helps another molecule or ion to move between the cellular compartments.
Transporters – an act or means by which a molecule or ion is moved across the membrane.

6.9 SELF ASSESSMENT QUESTIONS

6.9.1 Fill in the blanks:

1. _____act as a main substrate to the bacteroids, synthesized by the sucrose and delivered by the sieve tubes.
2. The ferredoxin molecule transfers a singleelectron molecule to _____-enzyme.
3. The _____catalyzes the process of nitrogen fixation.
4. The nitrogenase complex is composed of two components which are ____and ____-_.
5. _____act as an inhibitor for glutamate synthase.
6. The enzymes _____ reduce nitrite to ammonia.
7. A_____ is formed enclosing the bacteria within the cortical cells of roots through infection thread.
8. The enzyme _____ synthesizes glutamine through transferring NH_4^+ to the glutamate at the expense of ATP.
9. _____electrons are needed for nitrite reductase to convert nitrite into ammonia.
10. The enzyme _____ reduce nitrate into nitrite in plants and many other micro-organisms.

Answers Key: 1. Malate, 2. dinitrogenase reductase, 3. nitrogenase complex, 4. dinitrogenase reductase, dinitrogenase, 5. Azaserine, 6. nitrite reductases, 7. peribacteroid membrane, 8. glutamine synthetase, 9. 6, 10. nitrate reductases.

6.9.2 Multiple choice questions

1. Which gene is responsible for nodule formation?

a. Nod b. Nif
c. Fix d. All of the above

2. Dinitrogenase reductase and dinitrogenase, collectively makes up a complex which is known as

a. Nitrogen complex b. Nitrogenase complex
c. Nitrite complex d. Nitrate complex

3. How many ATP molecules are formed during nitrogen fixation?

a. 4 b. 8
c. 12 d. 16

4. What is the first step product of nitrogen fixation?

a. Ammonia b. Nitrate
c. Nitrite d. Atmospheric nitrogen

5. Which enzyme is responsible for Nitrate (NO3-) conversion to nitrite (NO2-)?

a. Nitrogen reductase b. Nitrogen dehydrogenase
c. Nitrate reductase d. Nitrate dehydrogenase

6. What is the central atom of seroheme made up of?

a.Co b. Cu
c. Mo d. Fe

7. Rhizobia are formed for the purpose of nitrogen fixation in what kind of plants?

a. Leguminous b. Non-leguminous
c. Agricultural d. All of the above

8. Bacteroids oxidize malate through which biochemical cycle?

a. Glycolysis b. Citric Acid Cycle
c. Gluconeogenesis d. None of the above

9. What are synthesized after the nodule formation?

a. Nodulins b. Early nodulins

c. Late nodulins d. All of the above

10. Glutamine synthetase transfers ammonia (NH4+) to the glutamate, forming the glutamine. The above reaction takes place by

a. Spontaneous process b. Non-spontaneous process

c. ATP expenditure d. Non-ATP expenditure

Answers to objective type questions

1. (a), 2. (b), 3. (d), 4. (a), 5. (c), 6. (d), 7. (a), 8. (b), 9. (c), 10. (c).

6.10 REFERENCES

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- Lehninger, A. L., Nelson, D. L., Cox, M. M., & Cox, M. M. (2005). *Lehninger principles of biochemistry*. Macmillan.
- Berg, I. M., & Tyrnoczko, I. L. (2007). stryer L, Biochemistry 6th ed. New York: W, H.

6.11 SUGGESTED READINGS

- Plant biochemistry Hans-Walter heldt (3rd edition)
- Principles of Biochemistry David L. Nelson and Michael M.Cox (6th edition)

6.12 TERMINAL QUESTIONS

1. What is the difference between asymbiotic and asymbiotic nitrogen fixation?

2. Describe the electron flow path within the nitrogenase complex in the process of nitrogen fixation.

3. What is nitrogen assimilation? What enzymes are involved in this process? Describe it through elaborated diagram.

4. Give a detail account about the nitrogen cycle.

UNIT-7 PLANT GROWTH REGULATORS

Contents:

7.1 OBJECTIVES

After reading this unit students will be able-

- To understand the metabolism of plants in light of different plant growth regulators i.e. auxin, gibberellin, cytokinin, ethylene and Abscisic acid.
- To know the history and physiological effects of different plant growth regulators
- To explain their mechanism of action or How they act in plants
- To know about their commercial use.

7.2 INTRODUCTION

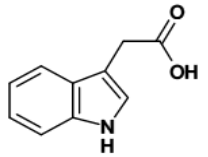
Growth in plants is defined as an irreversible increase in volume which is mainly driven by turgor pressure. In higher plants, regulation and coordination of metabolism, growth and morphogenesis often depend on chemical signals from one part of the plant to another. Julius von Sachs, the father of plant physiology, in later half of the 19th century also proposed that chemical messengers are responsible for the formation and growth of different plant organs. These chemical substances controlled the plant growth and development in extremely low concentration and are called as plant growth substances, growth hormones, phytohormones or plant growth regulators (PGRs).

7.3 PLANT GROWTH REGULATORS

According to Wareing and Phillips (1978), the term plant growth regulator covers the broad category of organic substances (other than vitamins and microelements) that in minute amounts either promote, inhibit, or modify physiological processes. The synthetic substances affecting various physiological processes in a plant are known as **plant growth regulators,** while the naturally occurring substances are known as **plant hormones/phytohormones**. The growth and development in plants is regulated by five major types of hormones or growth regulators. These growth regulators are grouped based on their function and chemical structure namely auxin, gibberellins, cytokinins, abscisic acid and ethylene.

7.4 AUXIN

Auxin was the first hormone discovered in plants as a growth promoting chemical in the tip of oat coleoptile. Auxin is the generic term used for growth substances that typically stimulate cell elongation, but it also causes a wide range of growth responses. A number of natural substances exhibit auxin activity but the first isolated and identified is Indole Acetic Acid (IAA), the most dominant one.



Indole-3-Acetic Acid
(an Auxin)

7.4.1 History

The presence of hormones in plants dates back to 1860, when Sachs suggested that there is some organ forming substance produced in the leaves which is translocated downwards towards the roots (polar transport). During the 19th century Charles Darwin and his son Francis Darwin investigated phototropism as well as geotropism of grass coleoptiles and demonstrated the presence of some signal which was transmitted to the lower region resulting in bending of coleoptiles towards unilateral light. The bending of coleoptiles was not caused if the tip of coleoptiles was covered or removed. These investigations laid the groundwork for Frits Went, who in 1926 obtained a diffusible growth-promoting factor from oat (*Avena sativa*) coleoptiles which was subsequently named auxin from the Greek word *auxein,* meaning *to increase, to enlarge or to grow*.

7.4.2 Physiological effects

Auxins are very widely used in plant tissue culture mainly in combination with cytokinins which promote the growth of callus. At cellular level, auxin control basic processes such as cell division and cell elongation. In organised tissues, auxins are involved in the establishment and maintenance of polarity and in whole plants their most marked effect is cell elongation.
Thus the physiological roles of auxin can be summarised as follows;

1) **Apical Dominance:** In most higher plants the growing apical bud inhibits the growth of lateral or axillary bud this phenomenon is known as apical dominance. After the discovery of auxin, it was found that IAA substitutes for the apical bud in maintaining the inhibition of lateral buds. The auxin that is transported basipetally (i.e. from tip to base) from the terminal bud is responsible for the outgrowth inhibition of axillary buds.
2) **Rooting:** Auxin induces both growth of pre-existing roots and adventitious root formation, i.e., branching of the roots. If the source of auxin is removed by trimming the tips of stems, the roots are less stimulated. In horticulture, auxins, especially naphthalene acetic acid (NAA) and Indole butyric acid (IBA), are commonly applied to stimulate root initiation in cuttings of plants. However, high concentrations of auxin inhibit root elongation and instead enhance adventitious root formation. Removal of the root tip can lead to inhibition of secondary root formation.
3) **Parthenocarpic or Seedless Fruits:** For fruit with unfertilized seeds, exogenous auxin results in parthenocarpy ("virgin-fruit" growth). Fruits form abnormal morphologies when auxin transport is disturbed.

4) **Flowering:** Auxin also exhibit a minor effect in the initiation of flowering and development of reproductive organs. In low concentrations, it can delay the senescence of flowers as well as fruits.
5) **Abscission**: **The falling of leaves, flowers and fruits is called abscission.** Auxin promotes the abscission of older leaves and fruits. The green lamina of young leaves produce auxin and the old leaves cannot produce sufficient auxin. The cell of the abscission layer secretes hydrolytic enzymes like cellulase and pectinase. These enzymes dissolve the cell wall. The organ detaches at this point and falls. Auxin inhibits the action of these enzymes and prevents abscissions. If auxin is applied on such leaves abscission is prevented. Similarly, application of auxins prevents abscission of flowers and fruits. NAA and 2, 4-D are the synthetic auxin used commercially to prevent premature fall of citrus and apple fruits.
6) **As herbicides:** 2, 4-D and 2, 4, 5-trichlorophenoxyacetic acid (2, 4, 5-T) were marketed as herbicides. 2, 4-D was the first widely used herbicide, and it is still so. It is easy and inexpensive to manufacture.
7) **Sex Expression:** Auxins change the sex ratio in some plants by increasing the number of female flowers and decreasing the number of male flowers. In dioecious species like Cannabis, genetically male plants produce exclusively male flowers but with the application of auxin it starts producing female flowers. This is the indirect effect of auxin by producing another hormone ethylene which develops more female flowers.

7.4.3 Mechanism of action

The main steps in auxin- (as well as other hormone-) signalling can be generally described as:
1. Initial perception of the hormone signal,
2. The signal transduction cascade, and
3. The final physiological response.

7.4.3.1 Auxin signal perception: Each target plant cell is presumed to possess receptors, which are able to detect hormonal signals and then to initiate the cascade of molecular events leading to the final physiological response. Receptor-like auxin-binding proteins have been identified especially Auxin-Binding Protein 1 (ABP1), is the major auxin-binding protein located in the lumen of endoplasmic reticulum but assumed to be active on the surface of cell.

7.4.3.2 Auxin signal transduction pathway(s): There is some indication that transduction of the auxin signal might be mediated by mechanisms based on a plasma membrane-located receptor a heterotrimeric G protein and phospholipase A2- or catalysed hydrolysis of specific membrane lipids.

Recent findings suggested the involvement of targeted protein degradation in auxin signalling. This mechanism is based on the regulation of the ubiquitin-conjugating pathway by auxin. Ubiquitin is a small and highly-conserved protein which facilitates protein degradation.

7.4.3.3. Auxin-regulated gene expression: Most auxin-controlled developmental processes involve modulation of gene expression in both positive (up-regulation) and negative (downregulation) manners. Several families of genes have been identified in a variety of different plants and organs that were rapidly induced after auxin treatment, namely *Aux/ IAA, GST* (glutathione-S-transferase), *SAUR* (small-auxinupregulated RNAs), *ACC* (aminocyclopropane carboxylic acid) synthase, *GH3* genes and many others. Fewer auxin-responsive down-regulated genes have been described and these were mostly identified in soybean hypocotyls.

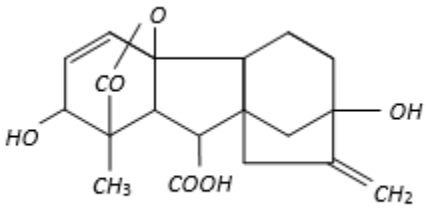
The main players in the control of transcription by auxin are two families of transcription factors: ARFs (auxin response factors), which can bind to the auxin response elements within auxin-responsive genes, and Aux/IAA proteins, repressors, the expression of which are auxin-regulated.

7.4.4 Commercial uses

1. **Rooting:** Besides indole acetic acid, some other synthetic auxins especially naphthalene acetic acid and indole butryic acid are widely employed in initiating early and vigorous rooting in plants which are propagated by cuttings.
2. **Prevention of Pre-Harvest Drop of Fruits:** Premature falling of the fruits can be checked by the application of auxins such as 2, 4-D and 2, 4 6-trichlorophenoxy acetic acid which delay the formation of the abscission layer.
3. **Parthenocarpy:** Parthenocarpic seedless fruits are obtained on commercial scale by the application of auxins in many plants.
4. **Inhibition of Buds to Prevent Sprouting:** Auxins like a-naphthalene acetic acid prolong the dormancy of buds in potato tubers and thus are very useful in preventing the eyes of potato tubers to sprout during storage.
5. **Shortening of Internodes:** In apples and pear the fruits are developed on dwarf spurs. Treatment of the other terminal shoots with a-naphthalene acetic acid prevents the elongation of their internodes so that they also become dwarf. The latter may also bear the fruits.
6. **Selective Weed Killers:** For instance 2, 4-dichIoro phenoxyacetic acid. (2, 4-D) is a potent weed killer and most of the broad-leaved dicotyledonous plants which occur as weeds are killed by this synthetic auxin, but usually the cereals (which are monocotyledonous) remain unaffected.

7.5 GIBBERELLINS

Gibberellins (GAs) are one of the longest-known family of tetracyclic diterpenoids plant growth substances that regulate various developmental processes, including stem elongation, germination, dormancy, flowering, flower development and leaf and fruit senescence.



Gibberellic acid

7.5.1 History

Gibberellins were first of all isolated from fungus Gibberella fujikoraii, which was found to be responsible for "bakanae" or "foolish seedling" disease of rice in Japan during early 1800s. Such rice plants were thin, pale green, spindle shaped, longer by 50% than the healthy plants, and were sterile. The disease was found by Hori (1918) and Kurosawa (1926) to be caused by a fungus, Gibberella fujikori. The fungus is the perfect stage of Fusarium moniliforme. Kurosawa also found that the sterile filtrate of the fungus also caused appearance of disease symptoms in uninfected rice seedlings. The active substance was separated and named gibberellin by Yabuta (1935). Yabuta (1938) also prepared crystalline form of gibberellins. GA3 was the first gibberellin to be structurally characterized. Until now 125 different gibberellins have been identified. Many of them occur naturally in plants and fungi.A single plant also possesses a number of gibberellins. This is in contrast to auxin, where a single natural hormone occurs. Gibberellins are synthesised in the apical shoot buds (young leaves), root tips and developing seeds. The precursors for their synthesis is mevalonic acid (derived from acetyl coenzyme A). Gibberellin transport occurs through simple diffusion as well as through conducting channels.

7.5.2 Physiological effects

1. Seed Germination: Certain light sensitive seeds e.g., lettuce and tobacco show poor germination in dark. Germination starts vigorously if these seeds are exposed to light or red light. This requirement of light is overcome if the seeds are treated with gibberellic acid in dark.

2. Dormancy of Buds: In temperate regions the buds formed in autumn remain dormant until next spring due to severe colds. This dormancy of buds can be broken by gibberellin treatment. In potatoes also, there is a dormant period after harvest, but the application of gibberellin sprouts the eyes vigorously.

3. Root Growth: Gibberellins have little or no effect on root growth. At higher concentration in some plants, however, some inhibition of root growth may occur. The initiation of roots is markedly inhibited by gibberellins in isolated cuttings.

4. Elongation of the Internodes: Most pronounced effect of gibberellins on the plant growth is the elongation of the internodes, so much so that in many plants such as dwarf pea, dwarf maize etc., they overcome the genetic dwarfism. For instance, the light grown dwarf pea plants have short internodes and expanded leaves. But, when treated with gibberellin the internodes elongate markedly and they look like tall plants.

It is considered that in such dwarf plants (i) the gene for producing gibberellin is missing, or (ii) the concentration of the natural inhibitors is higher. When external gibberellin is applied the

deficiency of the endogenous gibberellins is made good or the external gibberellin overcomes the effect of natural inhibitors which fall short.

Deepwater rice (Oryza sativa) is another notable example of pronounced effect of gibberellins on elongation of internodes so that its foliage may remain above water in the field.

Note: Partial submergence of rice plants is believed to reduce partial pressure of O_2 which triggers ethylene biosynthesis in submerged tissues. Ethylene in turn reduces the level of ABA (abscisic acid) which acts as antagonist of GA. Submerged rice tissues thus become more responsive to endogenous GA resulting in marked elongation of internodes).

5. Bolting and Flowering: In many herbaceous plants the early period of growth shows rosette-habit with short stem and cauline leaves. Under short days the rosette habit is retained while under long days bolting occurs i.e., the stem elongates rapidly and is converted into floral axis bearing flower primordia. This bolting can also be induced in such plants

e.g. (i) *Rudbeckia speciosa* (It is a Long Day Plant) by the application of gibberellin even under non-inductive short days.

(ii) In *Hyoscyamus niger* (also a Long Day Plant) gibberellin treatment causes bolting and flowering under non-inductive short days. While in Long Day Plants the gibberellin treatment usually results in early flowering, its effects are quite variable in Short Day Plants. It may either have no effect, or inhibit, or may activate flowering.

6. Parthenocarpy: Germination of the pollen grains is stimulated by gibberellins, likewise the growth of the fruit and the formation of parthenocarpic fruits can be induced by gibberellin treatment. In many cases e.g., pome and stone fruits where auxins have failed to induced parthenocarpy the gibberellins have proven to be successful. Seedless and fleshy tomatoes and large sized grapes are produced by gibberellin treatment on commercial scale.

7. Light Inhibited Stem Growth: It is common observation that the dark grown plants become etiolated and have taller, thinner and pale stems while the light grown plants have shorter, thicker and green stems, and it may be concluded that light has inhibitory effect on stem elongation. Treatment of light grown plants with gibberellin also stimulates the stem growth and they appear to be dark brown. In such cases the protein content of the stem falls while soluble nitrogen content increases probably due to more breakdowns of proteins than their synthesis.

It is considered that the light in some way lowers the level of endogenous gibberellins and inhibits the stem growth.

8. De novo Synthesis of the Enzyme-α-Amylase: One of the important functions of gibberellins is to cause de novo (i.e., a new) synthesis of the enzyme a- amylase in the aleurone layer surrounding the endosperm of cereal grains during germination. This enzyme brings about hydrolysis of starch to form simple sugars which are then translocated to growing embryo to provide energy source (Figure 7.1).

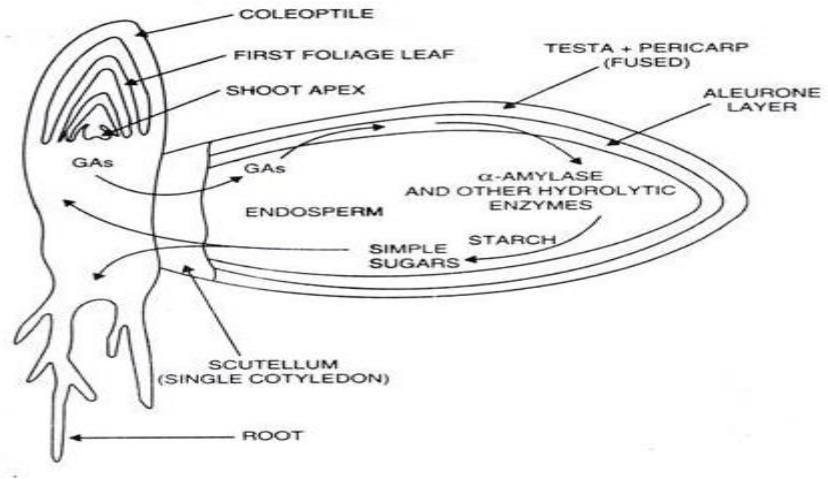


Figure 7.1 Mobilization of food reserve in germinating barley grain

7.5.3 Mechanism of action

The discovery of a soluble receptor, GA INSENSITIVE DWARF 1 (GID1) has led to many doubts that a membrane-bound receptor exists. GID1 was first identified in rice GID1s have a high affinity for bioactive GAs. GA binding to GID1 causes changes in GID1 structure, causing a 'lid' on GID1 to cover the GA binding pocket. The movement of this lid results in the exposure of a surface which enables the binding of GID1 to DELLA proteins. DELLA proteins, such as SLR1 in rice are repressors of plant development. DELLAs inhibit seed germination, seed growth, flowering and GA reverses these effects. DELLA proteins are characterized by the presence of a DELLA motif (aspartate-glutamate-leucine-leucine-alanine or D-E-L-L-A in the single letter amino acid code).

The first targets of DELLA proteins identified were PHYTOCHROME INTERACTING FACTORs (PIFs). PIFs are transcription factors that negatively regulate light signalling and are strong promoters of elongation growth. In the presence of GA, DELLAs are degraded and this then allows PIFs to promote elongation. DELLAs repress a large number of other transcription factors, among which are positive regulators of auxin, brassinosteriod and ethylene signalling. DELLAs can repress transcription factors either by stopping their binding to DNA or by promoting their degradation.

7.5.4 Commercial uses

1. Fruit Growth: Gibberellins when applied increases the number and size of several fruits, e.g., Grape, Tomato etc. The hormone creates more compartments by increasing the size of stalks so that fruits can grow in size. Size and shape of Apple fruits is enhanced commercially by applying the mixture of GA_4 and GA_7.

2. Parthenocarpy: Seedless fruits are commercially produced by application of gibberellins to un-pollinated flowers.

3. Malt: Gibberellins (e.g., GA_3) increase the yield of malt from barley grains.

4. Overcoming Dormancy: Gibberellins can be employed for breaking seed and bud dormancy. They induce germination of positively photoblastic seeds of Tobacco and Lettuce in complete dark.

5. Delayed Ripening: GA_7 delays senescence so that fruit can be left on the tree for longer period. It extends period of marketing. Ripening of Citrus fruits can be delayed with the help of gibberellins. This is useful in storing the fruits.

6. Flowering: Gibberellins can be used in inducing offseason flowering in many long day plants as well as plants requiring vernalisation.

7. Stem elongation: Spraying of sugarcane crop with gibberellins increases length of stem and yield of sugarcane.

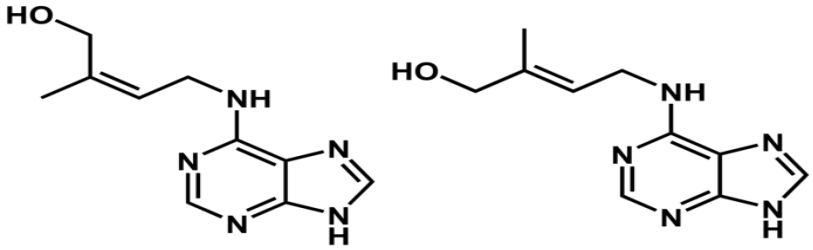
8. Early Maturity: Juvenile conifers sprayed with mixture of GA_4 and GA_7 reach maturity quite early resulting in early seed production.

7.6 GROWTH RETARDANTS

Many commercial compounds inhibit the synthesis of gibberellins. These inhibitors which are called **growth retardants**, include B-9, Cycocel (CCC), Phosphon D and Amo- 1618. Growth retardants inhibit stem elongation whereby producing stunted plants. Growth retardants are frequently sprayed on growing chrysanthemums to produce flowers with thicker, sturdier stalks.

7.7 CYTOKININS

Skoog and Miller (1965) have defined cytokinins as chemicals which, regardless of their activities, promote cytokinesis (cell division) in cells of various plant organs. Cytokinins are compounds derived from a nitrogen-containing compound (adenine). One cytokinin is 6-furfurylaminopurine (kinetin); other compounds derived from adenine with effects similar to those of kinetin, and certain compounds derived from another nitrogen-containing compound, urea, are conveniently referred to as cytokinins, although not all are natural products. Cytokinins are synthesized in roots, from which, like the gibberellins, they move upward in the xylem and pass into the leaves and the fruit. Required for normal growth and differentiation, cytokinins act, in conjunction with auxins, to promote cell division and to retard senescence, which, at least in its early stages, is an organized phase of metabolism and not just a breakdown of tissue.



cis-zeatin *trans*-zeatin

7.7.1 History

Coconut milk was discovered as an active stimulant of cell division by Van Overbeek *et al* in 1941. Later, in 1955 Carlos Miller *et al* isolated a *"cell-division-stimulating factor*" from yeast DNA and was named as kinetin because of its amazing power to stimulate cell division (cytokinesis) in the presence of an auxin. The first common natural cytokinin identified was purified from immature maize kernels and named 'zeatin'.Subsequently till now, many other compounds promoting cell division have been synthesized. Miller and his associates (1956) have grouped all such compounds including kinetin under a generic name kinin. D.S. Leetham (1963) of New Zealand proposed the term cytokinins for such substances. This term is the most acceptable one. Fairley and Kilgour (1966), however, prefer to use the term **'phytokinins'** for such substances in order to distinguish them from the peptide hormones of animal gastrointestinal tract.

7.7.2 Physiological effects

1. Cell division: Kinins are notable for their stimulatory effect on cell division. If a mixture of cytokinin and auxin is added to unorganized cells they will begin to differentiate. A high cytokinin to auxin ratio will lead to the formation of shoots, buds and leaves while a low cytokinin to auxin ratio will lead to root formation and equal ratio of both will result into the formation of callus.

2. Cell elongation: Besides auxins and gibberellins, kinetin also promotes cell elongation. Such promotion after kinetin treatment has been observed in tobacco pith cultures (Glasziou, 1957), tobacco roots (Arora et al, 1959) and bean leaf tissues (Powell and Griffith, 1960).

3. Root growth: Kinetin is capable of stimulating as well as inhibiting root development. Kinetins also induced increase in dry weight and elongation of the roots of lupin seedlings (Fries, 1960).

4. Shoot growth: Leafy shoots are initiated to develop when the amount of kinetin is increased. Bean seedlings, soaked in kinetin solution, also showed marked increase in dry weight and elongation of stem and petioles (Miller, 1956).

5. Organogenesis: The formation of organs, *i.e.* organogenesis is one of the effect shown by cytokinins in a variety of tissue cultures. The kinins also stimulate the production of buds in leaf segments of various plants such as *Bryophyllum sp and Begonia* sp. Cytokinins also bring about other morphogenetic responses in addition to the root and shoot differentiation such as maturation of proplastids into plastids and differentiation of tracheids.

6. Breaking dormancy of seeds: Cytokinins are effective in breaking seed dormancy in lettuce, tobacco, white clover and carpet grass. The seeds of parasites such as Striga asiatica require the presence of host plant for germination, but when treated with kinetin, the seeds germinate even in the absence of their host.

7. Delay of senescence (= Richmond-Lang effect): Richmond and Lang (1957) showed that the senescence in the detached leaves of Xanthium could be postponed for many days by kinetin

treatment. This effect of kinetin in retarding senescence (or ageing) is known as *Richmond-Lang effect.* It may, however, be emphasized that the cytokinin-induced delay in leaf senescence occurs only in detached leaves; cytokinins have little or no effect on senescence in attached organs. Leaf senescence is also delayed by the formation of adventitious roots. As the roots are rich in cytokinins, the transport of these cytokinins from roots to leaves could account for the delayed senescence.

8. Role in abscission: Cytokinins can accelerate as well as retard the process of abscission in leaf petioles depending on the site of their application (Osborne and Moss, 1963).

10. Effects on cotyledons: Cytokinins promote cellular division and expansion in cotyledons by inducing increase in wall plasticity that do not involve wall acidification. Cytokinins also increase the amount of sugars (especially glucose and fructose) in cells, which causes the osmotic influx of water and the resulting expansion of cytokinins treated cells in cotyledons.

7.7.3 Mechanism of action

The plant hormone cytokinin is perceived by membrane-located sensor histidine kinases. Arabidopsis (*Arabidopsis thaliana*) possesses three cytokinin receptors located in plasma membrane: ARABIDOPSIS HISTIDINE KINASE2 (AHK2), AHK3, and CYTOKININ RESPONSE1/AHK4. The ARR (A response regulator proteins) are transcriptional activators that carry MYB like DNA binding domains and glutamine Q' rich activating domains. There are 54 gene encode AHKs, AHPs and ARRs.

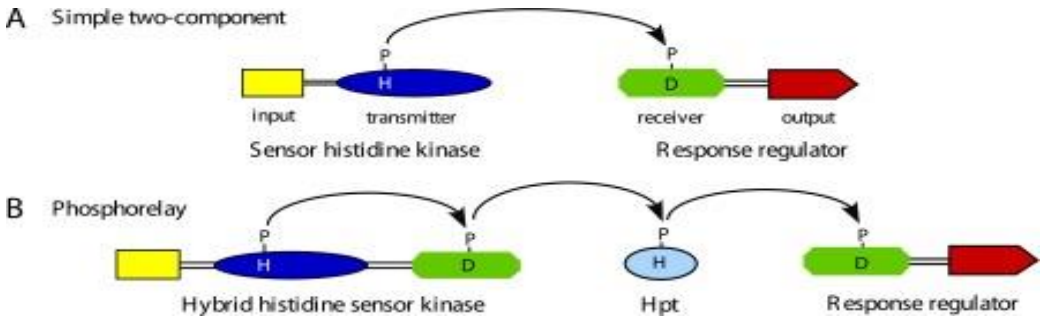


Figure 7.2 a two-component signalling pathway for cytokinin

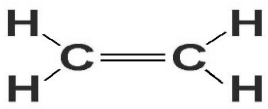
Cytokinin signalling is mediated by a two-component signalling pathway shown in the figure 7.2 a given above. In brief, cytokinin induces autophosphorylation of a histidine kinase (HK) protein, which results in the transfer of a phosphoryl group from a phospho-accepting histidine residue in the kinase domain to an aspartate residue. The phosphoryl is then transferred to a conserved histidine on a histidine phosphotransferase (HP) protein. From there, it is finally transferred to an aspartate in the receiver domain of a response regulator (RR).

7.7.4 Commercial uses

The commercial use of cytokinins on edible crops such as broccoli is banned in some countries. This is possible because any compound like cytokinin that resembles a nucleic acid component is automatically a suspected carcinogen. However, sometimes they are used commercially to maintain the greenness of excised plant parts, such as cut flowers.

7.8 ETHYLENE

Ethylene is known as a fruit ripening hormone produced in all parts of the plant. It is a gaseous molecule synthesized by most of the plants including angiosperms, gymnosperms, ferns, mosses and also even by fungi and bacteria. Ethylene gas accelerates respiration in fresh fruits and vegetables leading to maturity, senescence and softening of tissues. Ethylene accumulation can also cause yellowing of green vegetables. Apical tissues (shoot apex) and senescing tissues are rich sources. Physiological stresses including wounding, anaerobic conditions, flooding, chilling, disease and drought causes ethylene in action.



Ethylene

7.8.1 History

In 1864, it was discovered that gas leaks from street lights led to stunting of growth, twisting of plants, and abnormal thickening of stems (tripple response). In 1874 it was discovered that smoke caused pineapple fields to bloom. Smoke contains ethylene, and once this was realized the smoke was replaced with ethephon or naphthalene acetic acid, which induced ethylene production. In 1901, D. Neljubow realized that his dark-grown pea seedlings were short, fat and negatively gravitropic (**the tripple response**) because of a component in "laboratory air" which he subsequently identified as ethylene. Cousins (1910) first reported that ethylene occurred in plants. However, it was not until the latter half of the 20th Century, with the advent of gas chromatography, that ethylene achieved respectability as an endogenous regulator of plant growth and development.

7.8.2 Physiological effects

1. Stimulates fruit ripening: Ethylene stimulates the following processes that ultimately leads to fruit ripening.
(*a*) Breakdown of chlorophyll and synthesis of other pigments that causes change in colour of the fruit; for e.g., apples changing from green to red during ripening,
(*b*) Fruit softening due to breakdown of cell walls by cellulase and pectinase, and

(*c*) Conversion of starch and acids to sugars and ultimately ripening occurs.

2. Promotes flowering: Although ethylene inhibits flowering in most species but induces flowering in few plants including mangoes, pineapples and some ornamentals.

3. Promotes abscission: Abscission zone in leaves causes the increased production of ethylene which triggers the breakdown of middle lamella, thus leading to the initiation of abscission.

4. Induces epinasty: A physiologic response called epinasty is induced by ethylene that is released by the leaves when the roots are flooded. Flooded roots make ACC (immediate precursor of ethylene), the precursor to ethylene, and this is transported up the xylem stream to the leaf where it is converted to the gas hormone.

5. Controls stem elongation: Mechanical disturbances such as shaking enhance ethylene production several times, this effect is called **thigmomorphogenesis** that decrease stem elongation.

6. Sex expression. Both ethylene and gibberellins determine the sex of flowers in monoecious plants, i.e., plants having male and female flowers on the same individual.

7.8.3 Mechanism of action

Plants produce ethylene from methionine using a two-step biochemical pathway starting from S-adenosyl-L-methionine (SAM). SAM is converted to ACC by the enzyme ACC synthase (ACS) and ACC (1-aminocyclopropane-1-carboxylic acid) is then converted to ethylene by the enzyme ACC oxidase (ACO). The ACS and ACO enzymes are each encoded by a multigene family. Based on studies of the action of ethylene and its analogs, it is thought that ethylene binds to a metal-containing receptor before it exerts its physiological effects. The ethylene receptor is two-component signaling system, constituting N-terminal ligand-binding domain followed by a GAF domain and a histidine protein kinase domain. Some isoforms also have a C-terminal receiver domain, which is the second element of the two-component system. The ethylene responses are repressed by ethylene receptor signaling. This repression occurs in the absence of ethylene binding and is achieved through receptor activation of CONSTITUTIVE RESPONSE1 (CTR1), a serine/threonine protein kinase. CTR1 kinase activity negatively regulates the pathway (i.e., prevents downstream signaling). When ethylene binds to the receptors, ethylene receptor signaling ceases. Consequently, CTR1 is no longer activated and downstream ethylene signaling can proceed. Ethylene signaling involves a unique pathway, that consists of the following main steps: (i) ethylene is perceived by an ethylene receptor complex at the endoplasmic reticulum (ER) membrane; (ii) ethylene detection triggers cleavage of a key protein in the complex, ETHYLENE-INSENSITIVE2 (EIN2); (iii) the cleaved soluble portion of EIN2 is involved in repressing the translation of two regulatory F-box proteins, which would otherwise target two master transcription factors for degradation by the 26S proteasome; and (iv) rapid stabilization of the two transcription factors results in the regulation of gene expression.

7.8.4 Commercial uses

Ethylene is one of the most widely used plant growth hormones in agriculture. But being gaseous it is very difficult to be applied in the field. Therefore, some synthetic chemical compounds are available to overcome this problem. One such most commonly used chemical compound is ethephon (2-chloro ethylphosphonic acid) which is known by various trade names such as ethrel.

Commercial uses of ethephon (ethylene) are:

(i) The fruits such as cherries, grapes and blueberries are sprayed with ethephon to coordinate abscission, thereby allowing growers to harvest their crops in shorter periods of time or to minimize the harvesting period.

(ii) To induce fruit thinning (fruit drop) in cotton, cherry and walnut.

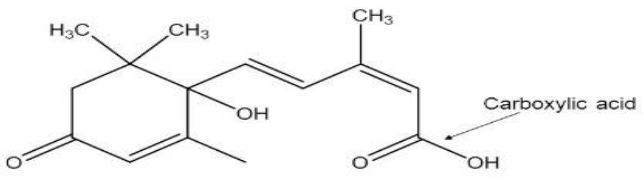
(iii) Aqueous solution of ethephon is also sprayed for de-greening of citrus fruits. It is also effectively used in synchronizing flowering and hastening abscission of flowers.

(iv)To promote formation (expression) of female flowers in cucumber, avoid self pollination and increase yield.

(v) Reverse measures are also employed on commercial scale to reduce rate of ripening, preventing over ripening to enhance post-harvest preservation of fruits and to increase longevity of cut carnations and other flowers by inhibiting or reducing the natural biosynthesis of ethylene.

7.9 ABSCISIC ACID

Abscisic acid (ABA) is a plant hormone that regulates plant growth, development, and various stress responses, so that it has also been called a stress hormone. It is a sesquiterpene (C_{15}) and has important roles in seed development and maturation, in the synthesis of proteins and compatible osmolytes, which enable plants to tolerate stresses due to environmental or biotic factors, and considered as a general inhibitor of growth and metabolic activities. ABA is synthesized in roots in response to stress and transported to the leaves though leaves are also capable of producing ABA.



Abscisic Acid

7.9.1 History

In 1963, Philip Wareing, isolated a compound named dormin in the buds of Acer pseudoplatanus. During the same period, Frederick Addicott discovered another substance that controlled the abscission of cotton fruits and named it as abscisin II. They also found that abscisin II also promotes leaf abscission in cotton seedlings and inhibits indoleacetic acid-induced growth of Avena coleoptiles. Later, dormin and abscisin II were found to be identical chemical compound and named abscisic acid (abbreviated as ABA).

7.9.2 Physiological effects

1. Stomatal closure: During drought, leaves synthesize large amounts of ABA which causes stomata to close. Thus, ABA acts as a messenger and enables plants to conserve water during drought. ABA-induced closure of stomata occurs within 1 to 2 minutes, this effect probably occurs independently of protein synthesis. The mechanism behind this is that, ABA probably produces its effect by binding to proteins on the outer surface of the plasmalemma of guard cells. This renders the plasmalemma more positively charged. There by stimulating transport of ions (especially K+) from guard cells to epidermal cells. The loss of these ions causes water to leave the guard cells (via osmosis) which then collapse, thus resulting in closure of stomatal aperture.

2. Delays seed dormancy: In many species, ABA delays seed germination. Similarly, in many other plants, the amount of ABA in their seeds decreases, when seeds germinate. Thus, it may be inferred that ABA controls seed dormancy in some cases. However, this conclusion may not be generalized, since germination of many seeds occurs without any changes in the amount of abscisic acid.

3. Controls bud dormancy: Bud dormancy was previously thought to be controlled solely by ABA. But besides ABA, bud dormancy is probably also influenced by cytokinins and IAA-induced synthesis of ethylene.

4. Counteracts the effects of other hormones. ABA counteracts the stimulatory/inhibitory effects of other hormones. For example,

(*a*) ABA inhibits cell growth promoted by IAA.

(*b*) ABA inhibits amylase produced by seed treated with gibberellin.

(*c*) ABA promotes chlorosis that is inhibited by cytokinins.

This may be due to the fact that ABA is a Ca^{2+} antagonist and its inhibition of the stimulatory effects of IAA and cytokinin may be due to its interference with Ca^{2+} metabolism. ABA often decreases gene activity, but there are instances of ABA stimulating genes. For example, ABA stimulates the synthesis of mRNAs for storage proteins in developing wheat grains.

7.9.3 Mechanism of action

ABA is a mobile signal, and its movement is an important mechanism of plant responses to drought stress which is mediated by influx and efflux carrier proteins. Several types of ABA receptors have been reported and, among them, PYR/PYL/RCAR receptors play a crucial role in ABA signaling (Figure 7.3).

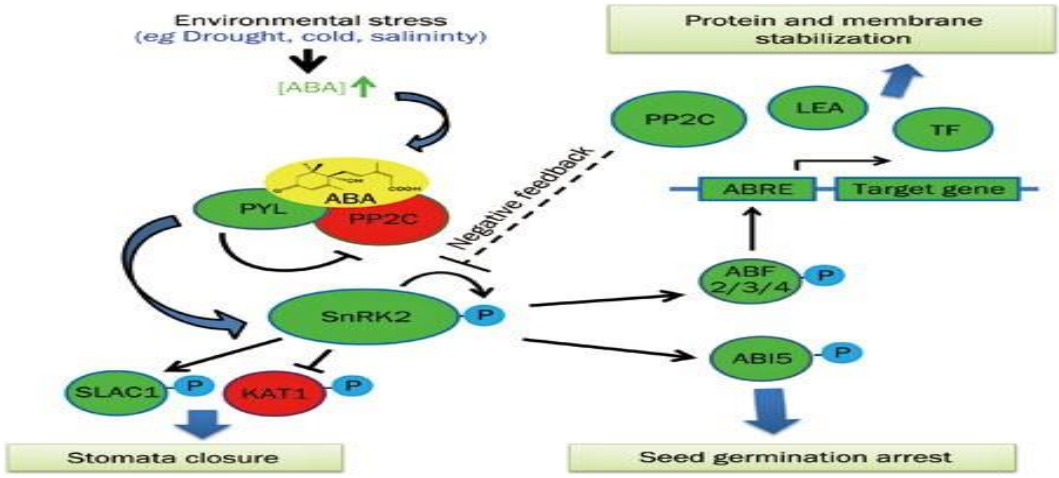


Figure7.3. ABA-mediated abiotic stress response.

Stress signals induced ABA accumulation that activates PYL ABA receptors to inhibit group A PP2Cs. PP2C inhibition in turn allows SnRK2 activation through autophosphorylation. Active SnRK2s mediate the ABA response through the phosphorylation of downstream targets. In guard cells, SnRK2s phosphorylate the SLAC1 and KAT1 ion channels, resulting in stomatal closure to prevent transpirational water loss. In seeds, ABI5 phosphorylation by SnRK2s leads to the inhibition of seedling growth. The phosphorylation of the AREB1 (ABF2), AREB2 (ABF4), and ABF3 transcription factors activates the transcription of target genes such as Late Embryogenesis Abundant (LEA)-class genes as well as transcription factors (TF) involved in stress tolerance. Transcriptional increases in the expression of group A PP2C genes may function as a negative feedback loop in the ABA response pathway by inhibiting SnRK2 activity.

7.9.4 Commercial uses

(i) It is used as an anti-transpirant. Application of minute quantity of abscisic acid to leaves reduce transpiration to a great extent through partial closure of stomata. It conserves water and reduces the requirement of irrigation.

(ii) Application of ABA in some short day plants results flowering under un-favourable photoperiods.

(iii) Use of abscisic acid in many stem cuttings promotes rooting.

(i) Abscisic acid is applied for prolongation buds dormancy, storage organs and seeds.

7.10 SUMMARY

Plant hormones (also known as phytohormones) are organic substances that are essential regulators of plant development beginning with seed germination and culminating in whole-plant senescence. Until recently it was generally believed that the five classes of compounds comprising auxin, gibberellins, cytokinins, ethylene, and abscisic acid, could account for most or all of the growth regulatory effects of plant hormones. A large number of related synthetic chemical compounds are used to regulate the growth of cultivated plants, weeds, and in vitro grown plants and plant cells. These human-made (synthetic) compounds are called plant growth regulators (PGRs).Plant hormones may be part of a signal-transduction pathway, or their presence may stimulate signal for stress responses. Plant hormones as signal molecules regulate cellular processes in targeted cells locally and when moved to other locations of the plant. They determine the formation of the root, stem, leaf, and flower and facilitate the shedding of leaves and the development and ripening of fruits. Hormones shape the plant and affect seed growth, time of flowering, sex of flowers, and senescence of leaves and fruits. They affect which tissues grow upward and which grow downward and even plant death. Hormones are vital to plant growth and lacking them, plants would be mostly a mass of undifferentiated cells. Plant hormones play important roles in diverse growth and developmental processes as well as various biotic and abiotic stress responses in plants. Endogenous regulations (e.g., biosynthesis, transport, redistribution, and conjugation of plant hormones) play a crucial role during the acclimation process against stress. Besides this, exogenous application of plant hormones has also been reported to enhance stress tolerance in plants. During the last decade, extensive work has been carried out to understand plant hormone-mediated enhancement in stress tolerance using physiological, biochemical, genetic, molecular, and genomic approaches for crop breeding and management.

7.11 GLOSSARY

Abscission: The dropping off of leaves, flowers, fruits, or other plant parts, usually following the formation of an abscission zone.

Anti-transpirant: Compounds applied to the leaves of plants to reduce *transpiration*.

Bioassay: The assessment of the effect of a chemical on an organism by comparison with the effects of standard substances of known concentration.

Callus: Growing mass of unorganized plant parenchyma cells.

Carotenoids: Yellow, orange, brown, or red lipophilic pigments that function as accessory photosynthetic pigments.

Chlorosis: Loss of green colour in foliage. Leaves appear typically pale or yellow in colour.

Coleoptile: A cylindrical sheath of tissue that encloses and protects young shoots of grasses and cereals during growth to the soil surface.

Dormancy: An inactive phase during which growth and developmental processes stop.

Receptors: They are dimeric transmembrane proteins that are thought to function as histidine kinases.

Signals: Internal and external factors that induce changes in cell structure and function.

Tissue culture: The growth of isolated plant cells or pieces of tissue under controlled conditions in a sterile growth medium.

7.12 SELF ASSESSMENT QUESTION

7.12.1 Multiple choice questions:

1. Hormone produced during water stress that brings about stomatal closure is:
(a) Abscisic acid (b) Ethylene
(c) Auxin (d) Gibberellin

2. The plant growth regulator that retards senescence is:
(a) Cytokinin (b) Gibberellic acid
(c) Indole acetic acid (d) Ethylene

3. Phytohormones are:
(a) Inorganic substances (b) Organic substances
(c) Both (a) and (b) (d) None of the above

4. Which of the following phytohormone was discovered first?
(a) Gibberellin (b) Cytokinin
(c) Auxin (d) Ethylene

5. The true natural auxin of higher plants is:
(a) indole-3-acetic acid (b) indole-3-acetaldehyde
(c) indole-3-pyruvic acid (d) indole-3-acetonitrile

6. Which of the following phytohormone was discovered first?
(a) Gibberellin (b) Cytokinin
(c) Auxin (d) Ethylene

7. Auxin is synthesized mainly in:
(a) Roots (b) meristimatic regions of the plant

(c) Shoots (d) none of the above

8. Which of the following is an antiauxin?
(a) 2,4-Dichlorophenoxyacetic acid (2,4-D) (b) 2,3,5-Triodobenzoic acid (TIBA)
(c) 2,4,6-Trichlorophenoxyacetic acid (d) all of the above

9. The phytohormone gibberellin was so named first by,
(a) Kurosawa (1926) (b) Yabuta (1936)
(c) Mitchell (1950) (d) Stodola (1955)

10. The fungus *Gibberella fujikuroi* causes backanae disease in:
(a) rice seedlings (b) maize seedlings
(c) wheat seedlings (d) none of the above

11. Chemical name of kinetin is:
(a) 5-furfurylamino purine (b) 6-furfurylamino pyrimidine
(c) 6-furfurylamino purine (d) none of the above
12. Fruit ripening hormone is:
(a) Ethylene (b) Auxin
(c) Kinetin (d) all of the above

13. 'Tripple response' of etiolated pea seedlings is caused by:
(a) ABA (b) IAA
(c) GA (d) Ethylene

14. *Avena-curvature* test for bioassay of auxin was developed by:
(a) Boysen-Jensen (b) F.W. Went
(c) Thimann (d) none of the above

15. Which of the following is considered as naturally occurring growth inhibitor in plants:
(a) IAA (b) ABA
(c) GA_1 (d) all of the above

16. Which of the following GA is immediate precursor of all other Gas in plants:
(a) GA_1 (b) GA_3
(c) GA_7 (d) GA_{12}

17. Which of the following is biologically most active natural cytokinin in plants:
(a) *Trans*-zeatin (b) *Cis*-zeatin
(c) Kinetin (d) All of the above

18. Immediate precursor of ethylene biosynthesis in plants is:
(a) Methionine (b) S-Adenosyl methionine
(c) 1-Aminocyclopropane-1-carboxylic acid (ACC) (d) None of the above

19. ABA occurs in plants predominantly in:
(a) Roots (b) Stems
(c) Mature green leaves (d) Flowers

20. ABA is a:
(a) Sesquiterpene (b) Diterpene
(c) Triterpene (d) Tetraterpenes

7.12.2 Fill up the blanks:

1. In plants _____amino acid is the precursor for the synthesis of auxin.
2. _____ is the richest source of gibberellin in higher plants.
3. Transport of auxin in plant is predominantly _____.
4. The phytohormone kinetin was discovered by _____.
5. _____is a potent weed killer.
6. Abscisic acid causes_____in water stressed plants.
7. _____ called as 'super-auxin' is a _____.
8. Deteriorative processes that naturally terminate functional life of plants are called as _____.
9. During polar auxin transport in plants_____proteins act as auxin efflux carriers.
10. Cytokinins are biologically_____in their bound form.
11. Prior treatment with_____greatly enhance the conversion of etioplast into chloroplast on exposure to sunlight.
12. Aqueous solution of_____is often sprayed on plants in field conditions to exert hormonal effects of ABA.
13. _____is the primary hormone causing abscission of leaves.
14. _____& _____are the inhibitors of ethylene biosynthesis.
15. 'Agent orange' the leaf defoliator used by USA in Vietnam War was _____& _____.

7.12.3 True or False:

1. The term auxin was coined by F.W. Went.
2. Ethylene is a gaseous hormone.
3. Glycine is the precursor of Indole acetic acid.
4. Gibberellic acid is involved in the regulation of florigen synthesis.
5. Genetic dwarfism can be nullified by spraying with zeatin.
6. GA9 is the gibberellin involved in flowering.

7. The first kinetin isolated by Miller was from herring sperm DNA.
8. *Agrobacterium tumefaciens* Ti plasmids t-DNA has genes for auxin and cytokinins.
9. In tissue culture high cytokinins to auxin ratio causes shoot differentiation.
10. Translocation of cytokinin is polar and takes place through phloem.
11. Adenine is the precursor of cytokinins.
12. Ethylene induces the aerenchyma formation in wet land species especially in rice.
13. Napthalene acetic acid is an artificial ripening agent.
14. Ethylene production can be detected by spectrophotometer.
15. Phytohormone cytokinin is helpful in making RNA and Protein.

7.12.4 Very short answer questions:

1. Name some synthetic auxins.
2. What is the role of auxin in sex expressions in plants?
3. What is seed dormancy and name the hormone involved in breaking seed dormancy?
4. What is the role of ABA as growth inhibitor?
5. How does ethylene play role in fruit ripening?
6. Define abscission and give the role of auxins in it
7. Explain: (a) Auxin Precursors (b) Anti-Auxin (c) Synthetic Auxins
8. Write note on cytokinins
9. Define plant growth regulators and name them?
10. Define the following abbreviations:

(a) IAA (b) 2,4-D (c) ABA (d) BA

7.12.1 Answer key: 1-a, 2-a, 3-b, 4-c, 5-, 6-a, 7-b, 8-b, 9-b, 10-a, 11-c, 12-a, 13-d, 14-b, 15-b, 16-d, 17-a, 18-c, 19-c, 20-a.

7.12.2 Answer key: 1-Tryptophan, 2-Immature seeds, 3-Polar, 4-Miller et al, 5-2,4-D, 6-stomatal closure, 7-Fusicoccin, fungal phytotoxin, 8-Senescence, 9-PIN, 10-Inactive, 11-Cytokinins, 12-Ethephon, 13-Ethylene, 14-AVG, AOA, 15-2,4-D & 2,4,5-T

7.12.3 Answer key: 1-True, 2-True, 3-False, 4-True, 5-False, 6-True, 7-True, 8-True, 9-True, 10-False, 11-True, 12-True, 13-False, 14-False, 15-True

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7.14 SUGGESTED READINGS

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2. Plant Physiology by Salisbury and Ross.
3. Fundamentals of plant physiology by V.K. Jain.
4. A text book of Plant physiology, biochemistry and biotechnology by S.K. Verma and Verma.

7.15 TERMINAL QUESTIONS

7.15.1 Short answer questions:

1. The role of ethylene and ABA is both positive and negative. Justify the statement
2. Define parthenocarpy. Name the plant hormone use to induce parthenocarpy.
3. Name a hormone which:

   a. is gaseous in nature
   b. is responsible for phototropism
   c. induces femaleness in flowers of cucumber
   d. is used for killing weeds
   e. induces flowering in long day plants
4. Write short note on;
   (a) Senescence (b) Cellular totipotency (c) Morphactins (d) Florigen
5. Name all the phytohormones with its structure.
6. Write practical applications of auxin.
7. Write a note on abscisic acid.
8. Write a short note on abscission layer.
9. Differentiate between ethylene and abscisic acid.
10. Write a note on commercial application of phytohormones.

7.15.2 Long answer question:

1. What are gibberellins? Discuss their biosynthesis and physiological role in plants.
2. Explain the role of auxin in plants? Describe their mechanism of action and biosynthesis.
3. Describe the biosynthesis, mechanism of action and practical applications of cytokinins.
4. What is a phytohormone? Give a comparative account of the physiological effects of auxins, gibberellins and cytokinins in plants.
5. Discuss the role of:
   a. Cytokinins in leaf senescence
   b. Auxins in apical dominanace
   c. Abscisic acid in abscission
   d. Gibberellic acid in dormancy
   e. Ethylene in fruit ripening

BLOCK-3
PLANT BIOCHEMISTRY AND STRESS PHYSIOLOGY

UNIT-8-CARBOHYDRATES AND LIPIDS

Contents:

8.1 *OBJECTIVES*

- To study about structure and functions of carbohydrates and lipids.
- Understand classification and types of carbohydrates and lipids.
- Identify properties of carbohydrates and lipids.

8.2 INTRODUCTION

Carbohydrates are major class of biomolecules and source of energy. Carbohydrates are also known as sugar and produced by the process of photosynthesis in plants. They are basically made up of C, H and O. Carbohydrates are major component of diet of living organisms and metabolized to release energy. Carbohydrate derivatives also contains N, S and phosphates. Carbohydrates combine with lipid to form glycolipid and with proteins to form glycoprotein. Beside carbohydrate lipids are another class of biomolecules which are crucial structural component of cell membrane of living cells. Lipids are generally represented by fats, oil and waxes. Several oil crops such as canola, mustard, sunflower, Seasame are commercially cultivated and represent major sources of oil.

8.3 CARBOHYDRATES

Carbohydrates can be defined as polyhydroxy aldehydes or polyhydroxy ketones or any compound which gives these on hydrolysis. Carbohydrates can be either aldoses or ketoses depending upon whether aldehyde group (-CHO) is present or ketonic group (-C=O)**.** The empirical formula of carbohydrates is $Cn(H_2O)n$ or $(CH_2O)n$ where value of n can be 3-7 for monomers.

Reducing and non reducing sugar

Sugar containing free aldehydic or ketonic group are known as reducing sugar. Reducing sugar exist in hemiacetal or hemi ketal forms. Such sugars can reduce Benedict solution, Fehlings solution. All monosaccharides (whether aldoses or ketoses) are reducing sugars. Disaccharides such as maltose and lactose are also reducing sugar. Sugar lacking presence of free aldehydic or ketonic group are called non reducing sugar.Non reducing sugar are found in either ketal or acetal forms. Non reducing sugardoesnot reduce Benedict solution and Fehlings solution. All polysaccharides are non reducing sugars, sucrose (which is a disaccharide) to also a non reducing sugar. Reducing sugars show the property of mutarotation whereas non reducing sugar doesnot show the property of mutarotation.

8.4 CLASSIFICATION OF CARBOHYDRATES

Based upon the monomeric units (number of monomers present) carbohydrates are classified into three categories (Figure 8.1):

a) Monosaccharide
b) Oligsaccharide
c) Polysaccharide

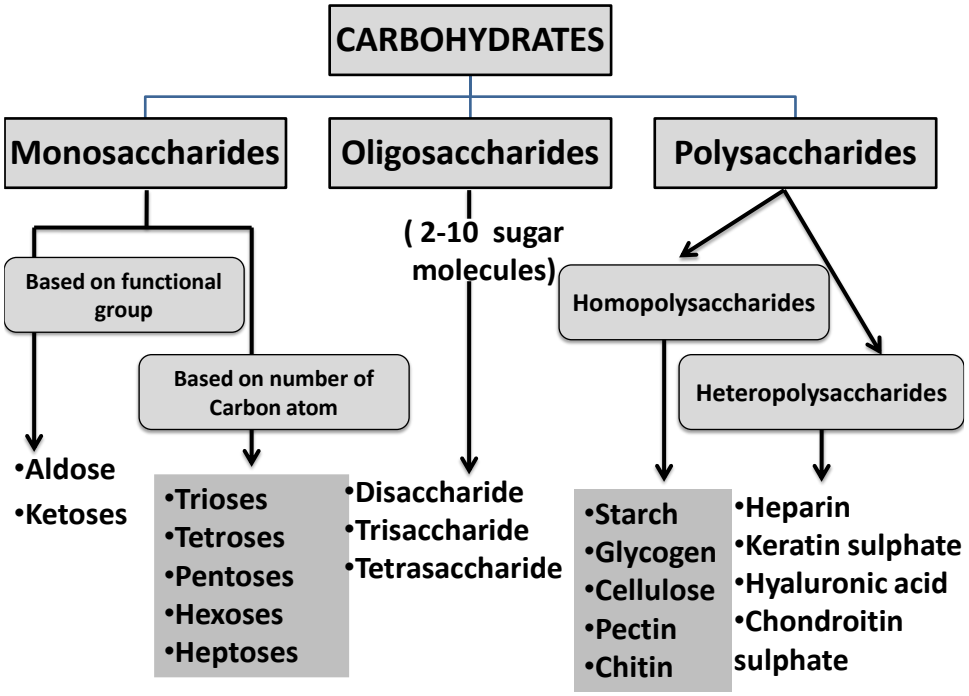


Figure 8.1: Classification of carbohydrates

8.4.1-Monosaccharides

They are simplest carbohydrates which cannot be further hydrolyzed (broken down) into smaller molecules.

Properties of monosaccharides:
1. Monosaccharides are crystalline and colourless.
2. At room temperature monosaccharides exist as solids.
3. Monosaccharides are extremely water soluble.
Although monosaccharides possess high molecular weightstill they are water soluble due to presence of multiple OH groups which helps in formation of intermolecular hydrogen bonding with water molecules making monosaccharides water soluble.
4. Monosaccharides can be aldoses (when aldehydic group is present)or ketoses (when ketonic group is present)

Different classes of monosaccharides have been identified depending upon number of carbon atom present. General empirical formula of monosaccharide is $Cn(H_2O)n$, where n represent number of carbon atom which can be from 3-7. This gives rise to five different structural types of monosaccharides (Table 8.2) which are trioses, tetroses, pentoses, hexoses and heptose.

Table 8.1 Different classes of monosaccharides based upon number of C atom

	Monosaccharide	Molecular formula	Aldoses	Ketoses
1	Trioses	$C_3H_6O_3$	Glyceraldehyde	Dihydroxy acetone
2	Tetroses	$C_uH_8O_4$	Erythrose	Erythrulose
3	Pentoses	$C_5H_{10}O_5$	Arabinose Ribose Xylose	Ribulose Xylulose
4	Hexoses	$C_6H_{12}O_6$	Glucose Mannose Galactose	Fructose
5	Heptoses	$C_7H_{14}O_7$	Glucoheptose	Sedoheptulose

Some common examples of biologically important monosaccharides

(a) Pentoses

Deoxyribose and ribose sugar are among the most common example of pentose monosaccharides. Deoxyribose sugar is found to be present as major structural component of DNA and similarly ribose sugar is structural component of RNA. Both the sugar differs structurally at Carbon number two (C-2). In ribose sugar OH group is present at C-2 whereas in deoxyribose sugar OH group is absent at C-2. Xylose is another example of pentose monosaccharide found in plant cells. This monosaccharide combines with xylan to form woody materials. Xylose is a reducing sugar due to presence of free aldehyde group. Like many other monosaccharides xylose also occurs in D and L forms, out of which D-xylose is found to occur naturally.

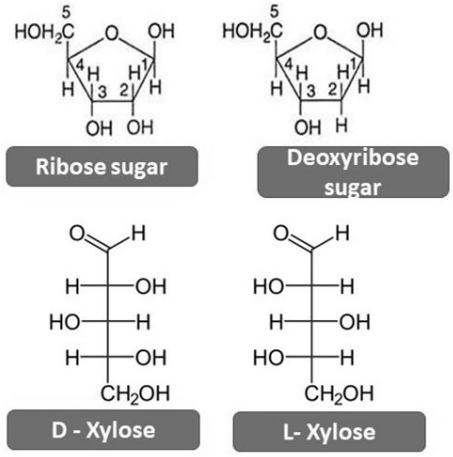


Fig. 8.2: Structure of some common pentose monosaccharides (sugar)

(b) Hexose sugar

Glucose, mannose, galactose, fructose are common examples of hexose sugar with general formula $C_6H_{12}O_6$ (Figure. 8.3). Glucose is a well known monosaccharide obtained through digestion of dietary carbohydrates and is main source of metabolic energy. Another

common hexose sugar is fructose which is a ketose monosaccharide. Fruit juices and honey are rich sources of fructose.Fructose binds to glucose to form the disaccharide sucrose. Both glucose and fructose are known to occur in D and L form. Glucose and fructose are also known as sweet sugar, fructose is much more sweet than glucose.

Galactose is an aldohexose, found in gum and pectin. Galactose is less sweet than other sugars. An important feature of galactose is it combines with glucose to from lactose.

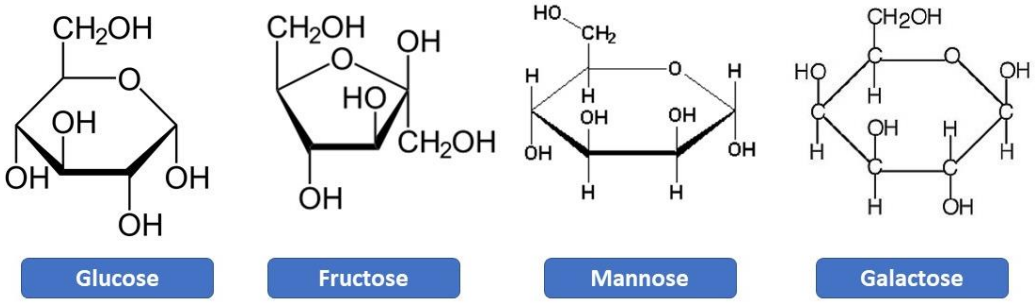


Fig. 8.3: Structure of some common hexose sugar

8.4.2-Oligosaccharides

Oligosaccharides are defined an sugars formed by polymerization of monosaccharide with a maximum of 9-10 monomeric units linked to one another by glycosidic bond, however most commonly occurring oligosaccharides have 2-6 monomers linked to one another. oligosaccharides are sweet in taste,crystalline in nature and water soluble. Oligosaccharides made up two monosaccharide units are called as disaccharides (common examples include sucrose, lactose and maltose), oligosaccharides made up three monosaccharide units are called as trisaccharides (e.g. raffinose), Oligosaccharides with four units are called as tetrasaccharides (e.g. stachyose) and so on. General empirical formula of disaccharides (Figure 8.4) is $C_n (H_2O)_{n-1}$ and that of trisaccharides and others is $C_n (H_2O)_{n-2}$.

Table8.2: Some common examples of disaccharides

Disaccharide	Molecular formula	Constituent monomer	Major source	Characteristic feature
Sucrose (cane sugar or table sugar)	$C_{12}H_{22}O_{11}$.	Glucose and fructose.	Sugarcane and beetroot.	Colourless, crystalline and water soluble. Non-reducing sugar. Doesnot exhibit mutarotation
Maltose	$C_{12}H_{22}O_{11}$.	Two units of glucose	Germinating seeds	Not readily found to occur naturally in nature. Obtained by degradation of starch It is a reducing sugar and exhibits mutarotation

				Less sweet in taste as compared to sucrose and fructose. Utilized in beer production
Lactose (Milk sugar)	$C_{12}H_{22}O_{11}$.	glucose andgalactose	Milk	Comparatively less soluble in water Less sweet in taste then sucrose. It is a reducing sugar Shows mutarotation

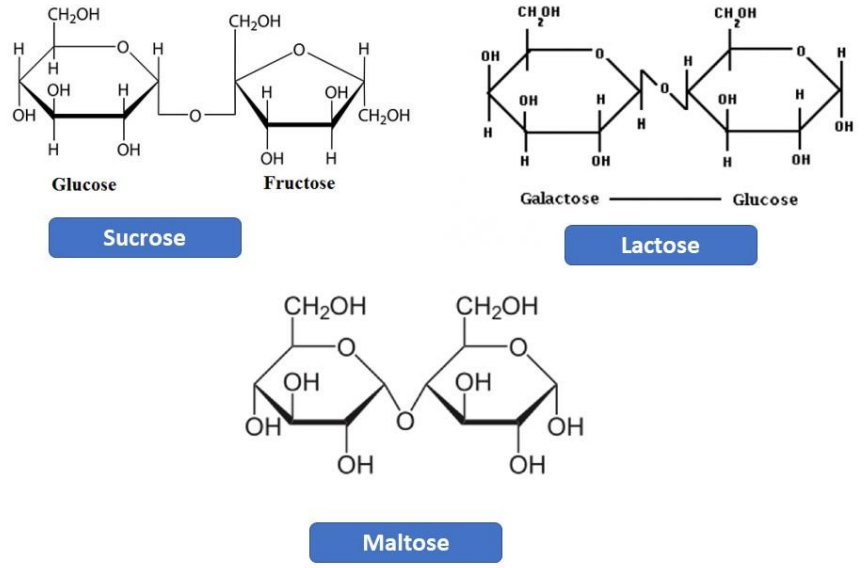


Figure 8.4: Structure of some common disaccharides

8.4.3 POLYSACCHARIDES

Polysaccharides are carbohydrates made up of hundreds and thousands of monosaccharide units. Based upon their biological function in living organisms polysaccharides are classified to be storage polysaccharides and structural polysaccharides. Storage polysaccharides are source of energy and major storage polysaccharides include starch in plants and glycogen in animals. Structural polysaccharides are involved in formation the cellular framework. For example cellulose is an important polysaccharides found in plants and is main component forming the framework of cell wall of plants. Chitin is another structural polysaccharide which is main component of cell wall of fungi. Polysaccharides are also classified as homopolysaccharides and heteropolysaccharides based upon their constituent monosaccharides. Polysaccharides which are made up of same monomeric monosaccharide unit, are called as homopolysaccharides and polysaccharides which are made up of different types of monosaccharides are called as heteropolysaccharides.

Some examples of Homopolysaccharides

(a) Starch

1. Starch is main food reserve (storage) material of plants stored in from of granules.
2. Starch is a non reducing sugar.
3. A molecule of starch comprises of two components ∝ - amylose and β-amylopectin (Figure 8.5 &8.6).
4. Amylose is linear part of starch in which C1 of one glucose unit in lined to C4 of another glucose unit by glycosidic linkage (C1-C4 glycosidic linkage).
5. Amylopectin possesses branched structure. At the point of branching occurs, C1 of glucose molecule of one chain in linked to C6 of glucose molecule of next chain by C_1-C_6 ∝glycosidic linkage.
6. Amylose in water soluble and amylopectin in water insoluble component.
7. A molecule of amylose can contain about 200-1800 α (1→4) bound glucose molecules and one molecule of amylopectin can contain 2000-3000 glucose molecules.

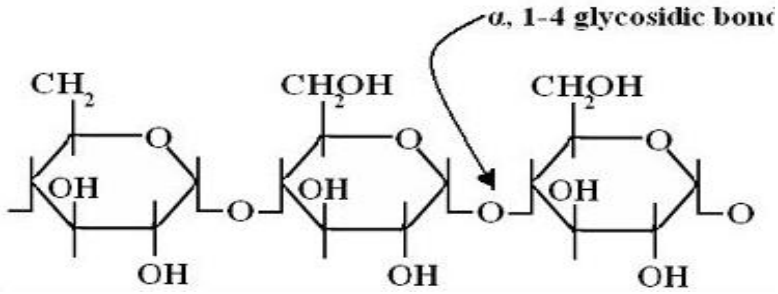


Figure.8.5. Structure of amylose

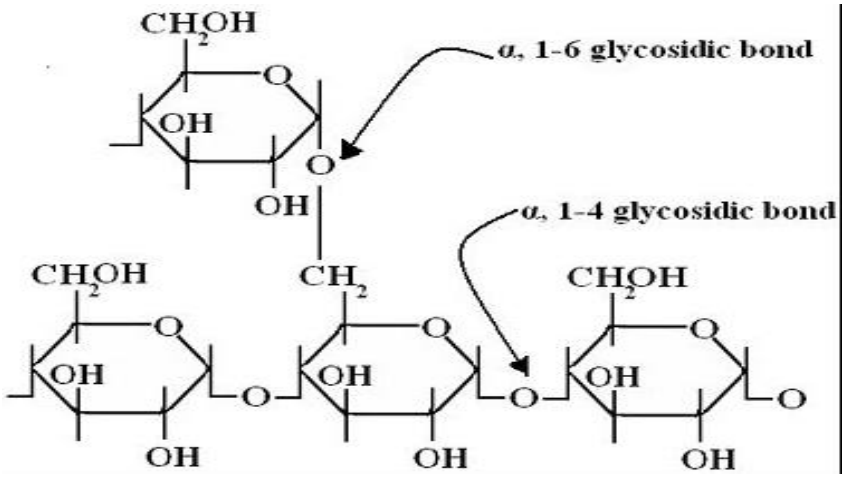


Figure.8.6. Structure of amylopectin

(b) Glycogen

1. Glycogen is reserve food material of animals made up of large number of glucose units.
2. Molecular formula of glycogen in $(C_6H_{10}O_5)_n$
3. Glycogen is water soluble.
4. Glycogen possesses branched structure similar to amylopectin.
5. Glycogen differs from amylopectin as it possesses much more branched structure.
6. In linear part of glycogen glucose units are linked to one another by ∝ (1-4) glycosidic linkage and the point from where branching occurs C6 of glucose of one chain is linked to C1 of glucose present in adjacent chain.
7. Glycogen is stored in liver.

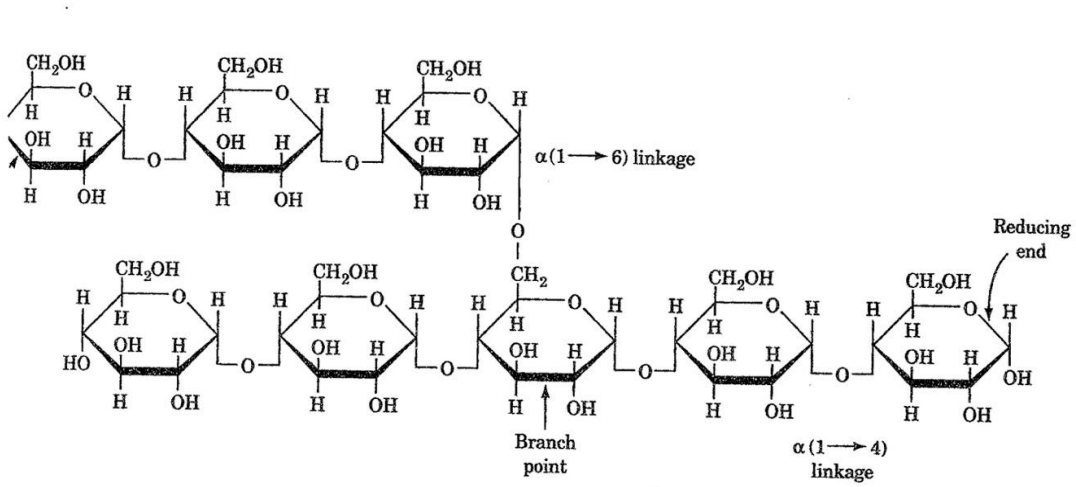


Figure.8.7. Structure of glycogen

(c) Cellulose:

1. Cellulose is most abundant molecule present on earth and major structural component of plant cell wall.
2. Cellulose is linear (unbranched) polymer made up of 6000-1,00,000 β-glucose units.
3. Molecular formula of cellulose is $(C_6H_{10}O_5)n$.
4. Cellulose is a non reducing sugar.
5. In a cellulose polymer C1 of β- D.glucose is linked to C4 of another glucose through β-D.glycosidic linkage.

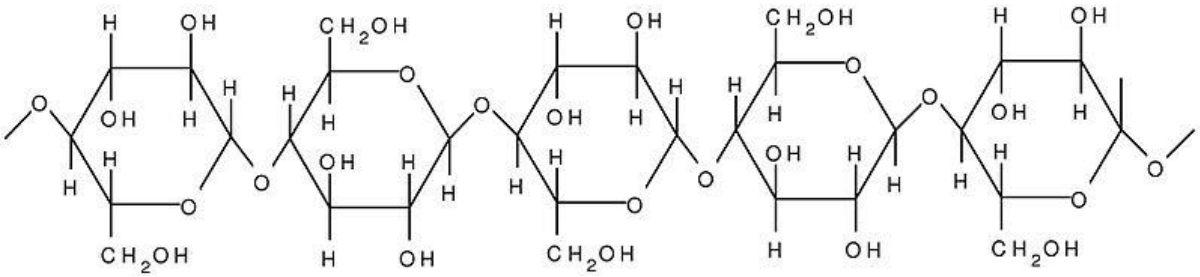


Figure8.8. Structure of cellulose

Some examples of Heteropolysaccharides

1. **Hyaluronic acid**: It is a disaccharide made up of repeating units of D – Glucuronic acid and N- Acetyl glucosamine linked by β - 1,3 linkage. Repeating disaccharide molecules are linked to one another by β-1,4-linkage. Hyaluronic acid acts as shock absorber.

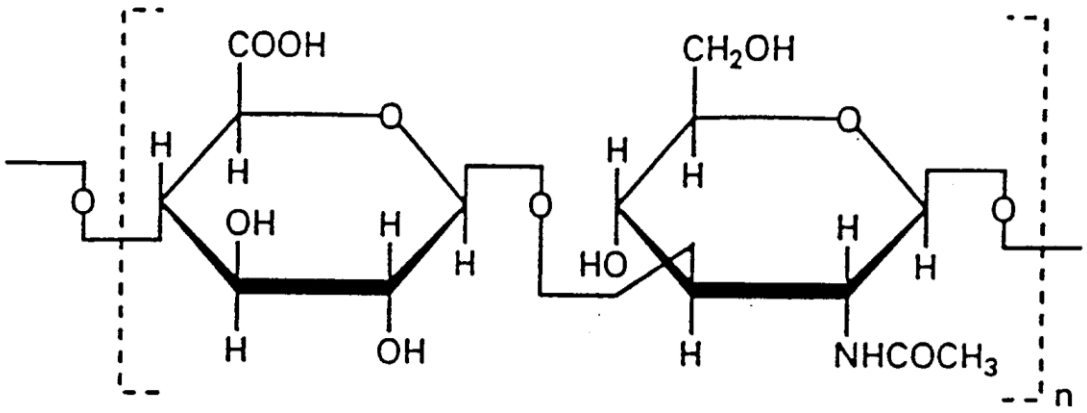


Figure.8.9. Structure of Hyaluronic acid

2. **Chondroitin sulphate**: Chondroitin sulphate is an important component of cartilage and is made up of alternating units of D-Glucuronic acid and N-Acetyl –D-galactosamine. Chondroitin sulphate possesses similar kind of linkage as found in hyaluronic acid. Chondroitin sulphate in presence of water swells forming a gelatinous matrix which functions as a good lubricant.

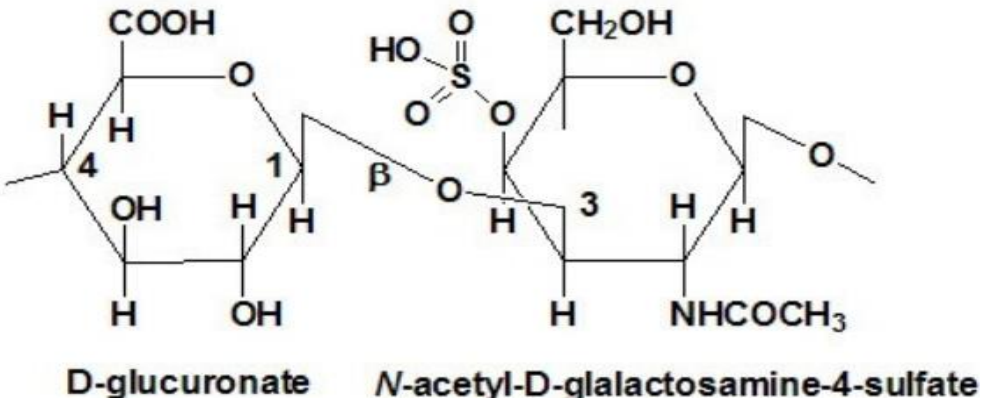


Figure8.10. Structure of Chondroitin sulphate

3. **Heparin**: Heparin consists of monomeric units of D-glucuronate sulphate / L-idurunote sulphate and N-sulphoglucosamine –6-sulfate which are linked by α (1 → 4) glycosidic bonds (Fig. 3.22). It is present in liver, lungs, spleen, monocytes etc.

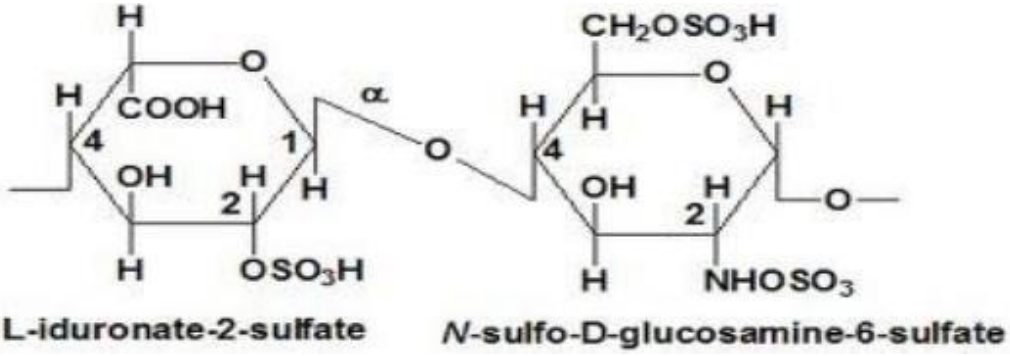


Figure8.11. Structure of heparin

8.5 STEREOISOMERISM

Many carbohydrates possess same number of atoms and similar group, still they are different compounds due to differential arrangement of functional groups. This phenomenon is called as stereoisomerism and the respective isomers are called as stereoisomers. Stereoisomers are typically defined as compounds with same structural formula but different spatial arrangement. The number of stereo isomers depends upon presence of asymmetric carbon atom. Asymmetric carbon atom is an atom to which four different groups are attached. Due to presence of chiral carbon atom these sugars are optically active and exist in two enantiomeric forms. Glyceraldehyde, which in simplest carbohydrate is taken as standard or reference to assign respective configuration to sugars.

D and L Configuration

A monosaccharide in which OH group at last chiral carbon is present toward right hand side is assigned D and the monosaccharide in which OH group on last chiral carbon atom in on left side are assigned L configuration. Fig.3.1 represents D and L form of glucose.

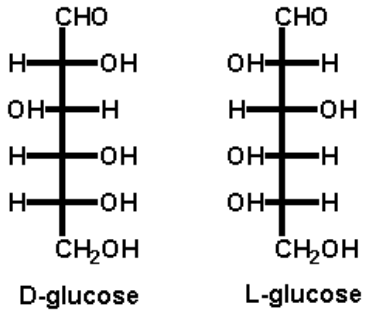


Figure 8.12 D and L configuration of glucose

(+) and (-) configuration

A compound having a asymmetric carbon atom is optically active, i.e when a plane polarized light is passed through a solution of optically active compound the light is rotated either in left or right direction. Sugars which rotate plane polarized light toward right are called on dextrorotatory and are represented by (+) and sugars which rotate plane polarized light in left direction are called levorotatory and are represented by (-).

Enantiomer and epimer

Enantiomer is one of the two stereoisomer that are mirror image of one another. Epimers are those sugar which differ from its isomers in configuration at only one asymmetric carbon. Example: - D-glucose and D-galactose contains four asymmetric groups, and that all these groups are identical except the configuration at C-4.

8.6 GENERAL PROPERTIES OF CARBOHYDRATES

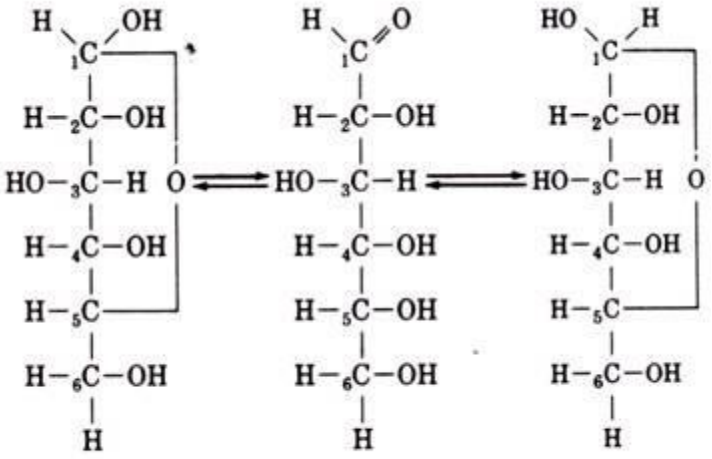
1. Carbohydrates are major class of biomolecule composed of C, H and O.
2. Carbohydrates are hydroxy (Generally polyhydroxy) aldehydes or ketones.
3. Carbohydrates derivatives also contain P, N and S.
4. Carbohydrates combine with proteins and lipids to form glycoprotein and glycolipids respectively.
5. Carbohydrates are found to exist as straight chain compounds or in closed ring structure.
6. Reaction between aldehyde or ketone part of carbohydrates with alcohol produces derivatives called hemiacetals and hemiketals which results in ring formation.
7. Monosaccharides are easily oxidized by oxidizing agents. Oxidation of glucose with Tollens reagent or Fehling's solution result in formation of glycolic acid.

8. Hydroxyl group of alcohols in carbohydrates can be converted to ester by treatment with acetylating agent.
9. Several methylating agent react with carbohydrate to form glycosides which can further lead to formation of tetra methyl esters.
10. Many microbial species are known to ferment monosaccharides to produce useful products such as acids, alcohols, etc.

8.7 STRUCTURE OF CARBOHYDRATES

Chain and Ring structure of monosaccharides :-

There are many simple sugars which can exist in chain (open) form or in ring form. Formation of ring structure in favored when these sugars are present in aqueous medium. All the sugars which undergo the process of ring formation almost follow a similar mechanism. The best example to study ring formation is that of glucose in which oxygen present on C-5 gets linked to C of Carbonyl group (i.e C-1) and hydrogen atom from C-5 is transferred to carbonyl oxygen to form OH group resulting in C1-C5 linkage which converts open chain glucose into ringed form. When the hydroxyl (OH) group and $–CH_2OH$ group are on opposite side α-glucose is produced and when both hydroxyl as well as CH_2OH group are on same side β-glucose is formed. Such isomers which differ only in configuration around carbonyl carbon are known as anomers.



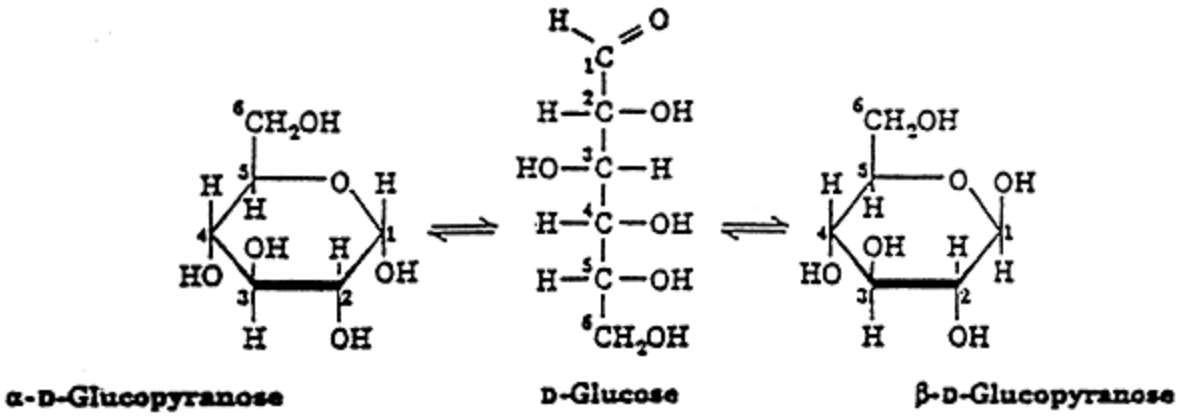


Figure 8.13 Chain and ring form of glucose

Glucose in solutions is mainly found in ring form due to formation of intramolecular hemi acetal. A hemi acetal is a structure formed when an aldehydic or ketonic group of a molecule combines (condenses) with hydroxyl (alcoholic) group present in the same molecule. Asymmetric C-1 allows glucose to exist in two isomeric forms called as α and β. α- glucose is represented by presence of OH group at C-1 on left side and β-glucose contains OH group at C-1 on right side.

Sugars with a six membered ring are also known as pyranoses. This is simply because 6-membered ring (in glucose) is similar to pyran both having a ring made up of five carbon and one oxygen atom. Presence of pyranose ring is commonly found in hexose sugars. Glucose is most common pyranose sugar and its α and β forms are also called as α-D-glucopyranose and β-D-glucopyranose respectively. Similarly, sugars with five membered ring are called as furanoses. This is due to similarity of five membered ring of sugar with furan ring, both have ring structure with four carbon and one oxygen atom. Fructose exists as pyranose sugar in free solution but when present as a monomer in sucrose it exists in furanose form.Similarly galactose can also exist in pyranose or furanose form.

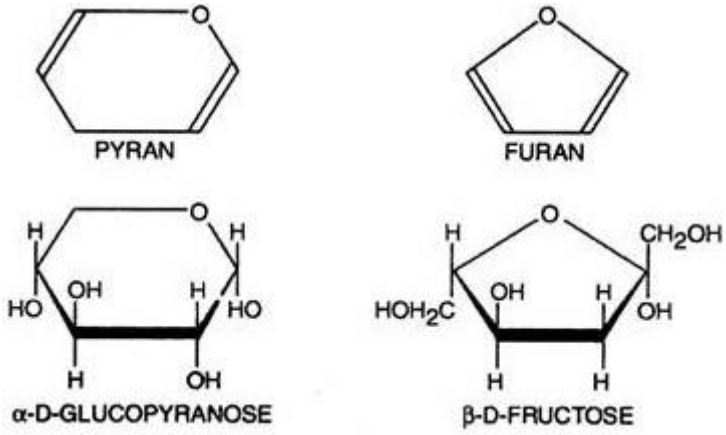


Figure 8.14. Similarity of six and five membered monosaccharide to pyran and furan ring

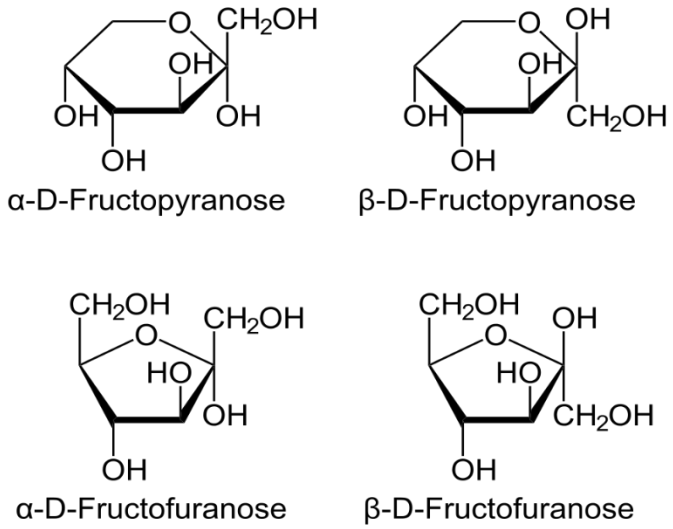


Figure 8.15. Pyranose and furanose forms of fructose

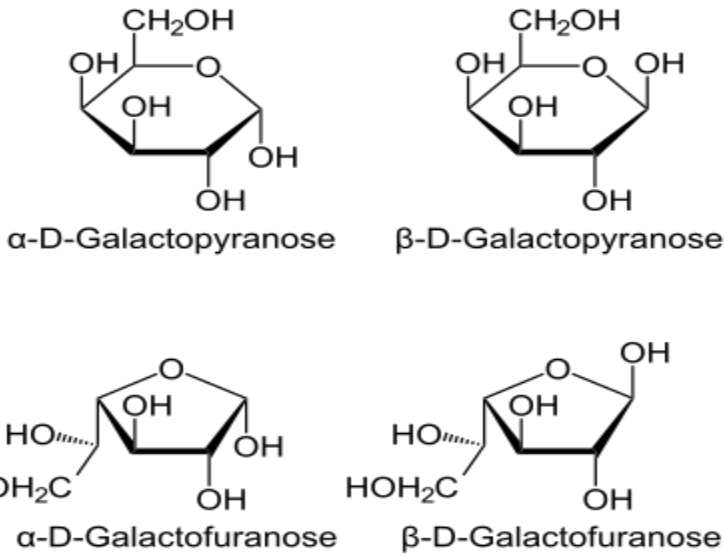


Figure 8.16. Pyranose and furanose forms of galactose

8.8 LIPIDS

Lipids are among the major class of biomolecules found to be present in all living organisms. The term lipid is derived from Greek word "lipos" that means Fat. Bloor (1943) for the first time utilized the term lipid. Lipids are considered as main storage from of energy and are involved in large number of biochemical reactions with several biological function. Lipids are also structural component of living cells. Lipids are chemically made up of C, H and O. Carbohydrates are also made up of C, H and O however concentration of O is lipids is comparatively low as compared to carbohydrates.

8.9 CLASSIFICATIONOF LIPIDS

Depending upon the nature of these molecules lipids can be classified in the three main categories

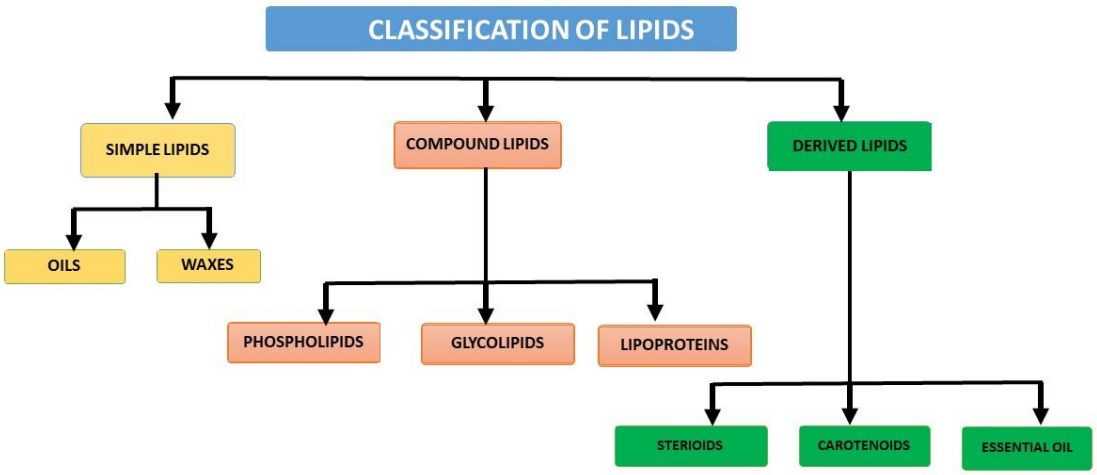


Fig. 8.17: Classification of lipids

1. Simple lipid :

Simple lipids are composed of fatty acid esters and alcohols. Fats/oils and waxes are the two types of commonly known simple lipids. Waxes are regarded as simplest known fatty acid esters found in nature. Based upon chemical structure waxes are esters of long chain (C_{14}-C_{16}) saturated and unsaturated fatty acids with long chain (C_{16}-C_{30}) alcohols. Due to their water repelling property, waxes are secreted by certain skin glands to protect hair and skin as secretion of waxes provides lubrication to skin. Leaves of some plants possess coating of wax for eg. leaves of rhododendron and other tropical plants, presence of wax coating over the surface of leaves protects against excess evaporation and also provides protection against parasites.

Table 8.3: Comparative analysis of fats/oil and waxes

	Fats and oil	Waxes
1.	Fats are solid at room temperature whereas oils are liquid.	Waxes are solids.
2.	Chemically composed of glycerol and three fatty acids.	Chemically composed of long chain alcohol and fatty acid.
3.	Fats and oil undergo rancidity.	Waxes do not undergo rancidity.
4.	Fats and oils are digester by lipase.	Waxes are not digested by lipase.
5.	Fats and oils are nutrients and source of energy for animals.	Waxes are not utilized by humans as source of nutrition.

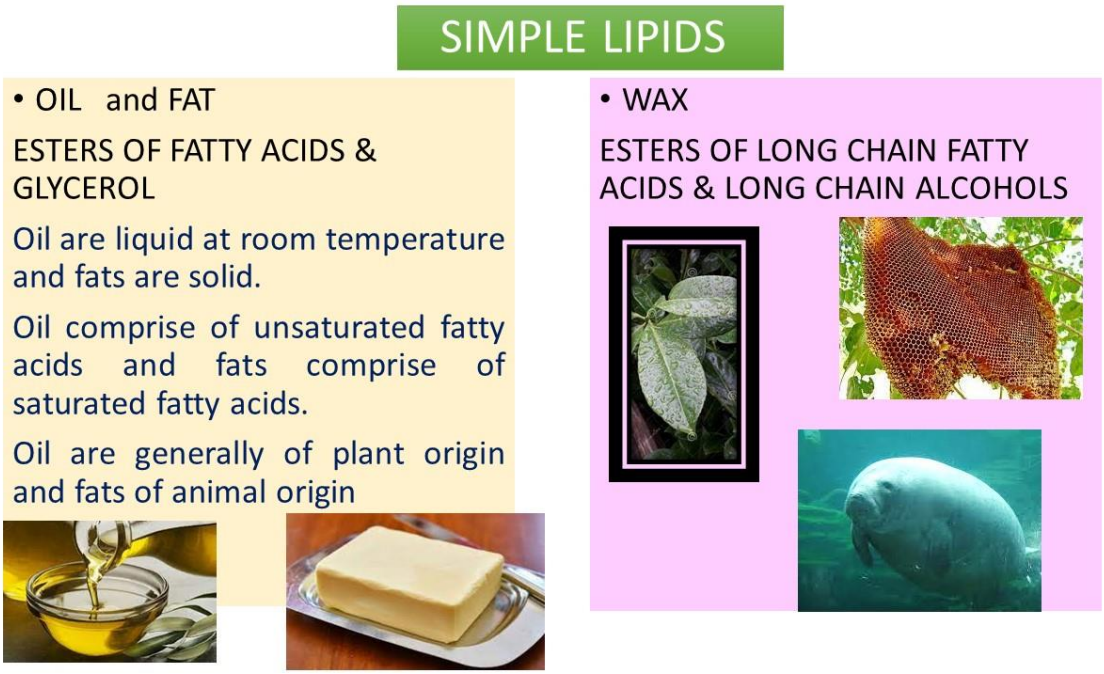


Figure 8.18: Summary of simple lipids

2. Compound lipids:

Compound lipids are those which contain on additional group to normal structure of a lipid i.e., an additional element, molecule is present along with fatty acid ester and alcohol. Compound lipids are also known as complex lipids. The additional group present in compound lipids can be a protein, nitrogenous base, carbohydrate, etc. Depending upon nature of additional group present compound lipids are classified into different groups.

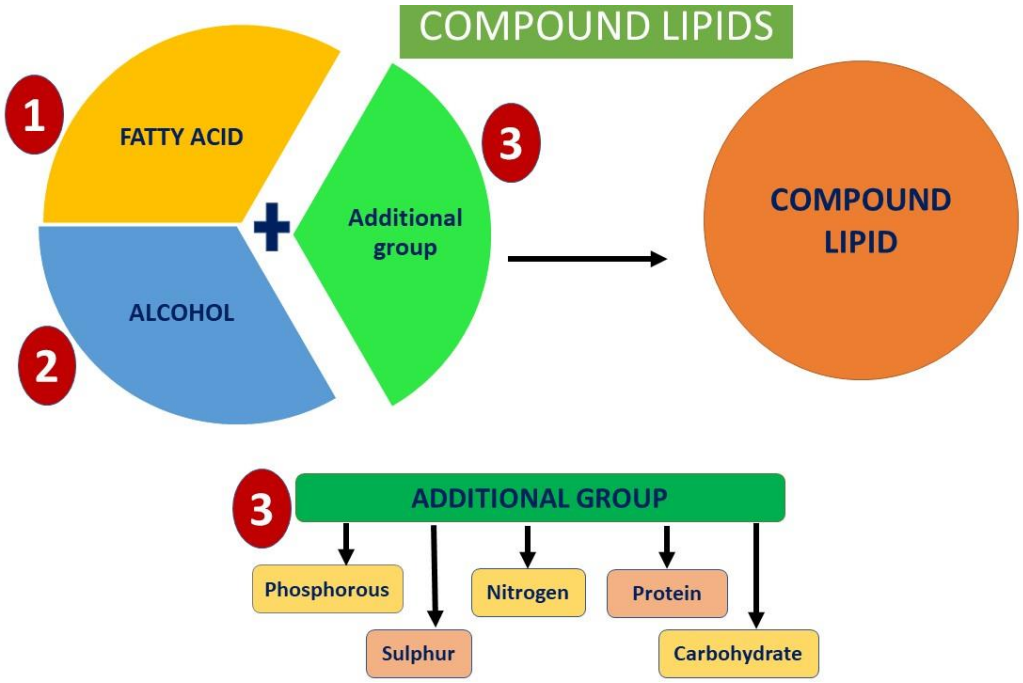


Figure 8.19: Organization of compound lipids

Table 8.4: Different types of compound lipids based on nature / type of additional group present:

	Type of compound lipid	Additional group present	Characteristic feature
1.	Phospholipids	Phosphoric acid (Phosphate)	Phospholipids can be (a) glycerophospholipids if alcohol present is glycerol. e.g. Lecithin, Cephalin (b) Sphingophospholipids if alcohol present is sphingosine ex. Sphingomyelin
2.	Glycolipids	Carbohydrate	Known as glycosphingolipids since they contain sphingosine as alcohol. e.g. Cerebrosides, Gangliosides.
3.	Lipoprotein	Protein	High density lipids (HDL), Low density lipids

3. Derived Lipids:

Derived lipids are obtained as hydrolytic product of either simple or compound lipids. Steroids, essential oil, carotenoids are common examples of derived lipids.

Saturated and Unsaturated fatty acids

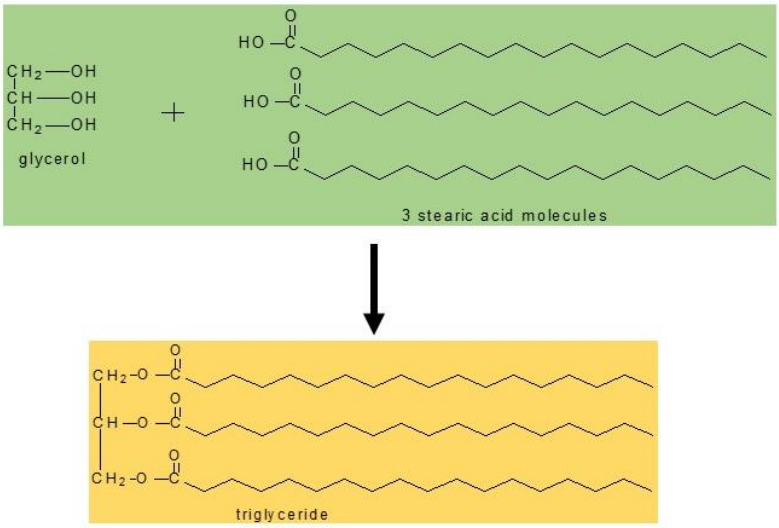
A compound is said to be saturated when it contains only single bonds between C atoms whereas unsaturated compounds are those which contains one or more double bonds. Lauric acid present

in coconut oil, myristic acid present in butter, Palmitic and Stearic acid found in fats and oil are commonly known saturated fatty acids. Unsaturated fatty acids contain double bonds between C atoms. Depending upon the number of double bonds unsaturated fatty acids can be monounsaturated when a single double bond is present or can be polyunsaturated when more than one double bonds are present in vegetable oil, linoleic acid found in soyabean and canola seeds and oleic acid present in olive oil are common examples of unsaturated fatty acids.

8.10 COMPOSITION

General organization of a lipid molecules

Lipid molecules are made up of fatty acid esters and alcohol. Fats and oils comprises of one glycerol molecule and three fatty acids linked through esterification.

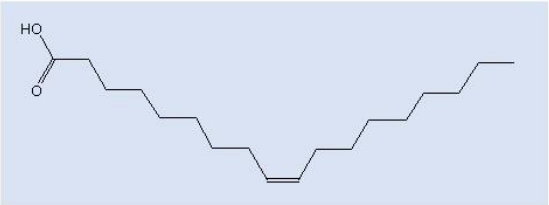


8.11 PROPERTIES WITH REFERENCE TO FATTY ACIDS

8.11.1 Oleic acid

Oleic acid is mono unsaturated omega $^{-9}$ fatty acid with molecular formula $C_{18}H_{34}O_2$. Oleic acid is naturally found in animal and vegetable fats and in oils. Olive, Palm, Peanut and Sunflower oil are common sources of oleic acid.Oleic acid does not normally occur in free state instead it is found as ester of glycerol. Oleic acid undergoes the reaction of carboxylic acids. Oils containing oleic acid are recommended to replace saturated fats in diet because it is expected that oleic acid might improve heart condition through decreasing the concentration of cholesterol and also reducing inflammation (serine oleic acid also acts as anti-inflammatory agent).

Molecular Mass	282.47g/mol
Density	895kg/m^3
Solubility	Insoluble in water soluble in ethanol.
Melting Point	13-14°C



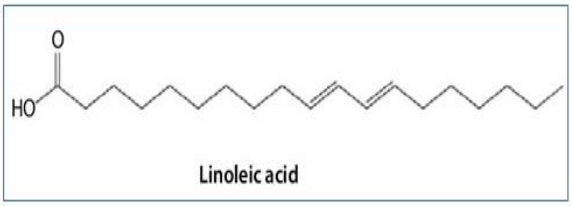
Uses:

1. Oleic acid is a component of many food in from of its triglycerides.
2. Being a part of animal fat and vegetable oil, it is a regular component of human diet.
3. It acts as emulsifying agent.
4. It is utilized as solubilizing agent in aerosol products.
5. Oleic acid is considered to play a role in blood pressure reduction.
6. Oleic acid has been reported to be anti-apoptotic and anti-inflammatory agent.

8.11.2 Linoleic acid

Linoleic acid is polyunsaturated essential fatty acid fount in various plant oil. Linoleic acid is an omega-6 fatty acid. Chemical formula of linoleic acid is $C_{18}H_{32}O_2$.

Molecular weight	280.44 g/mol
Density	0.9 g/cm
Solubility	Insoluble in water, soluble in organic solvent
Melting point	-5 °C



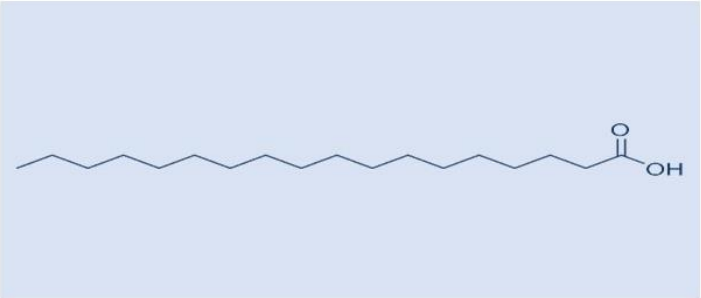
Uses:

1. Utilized in synthesis of prostaglandins.
2. It is a component of quick drying oils used in oil paints and varnishes.
3. Linoleic acid is also a surfactant.
4. Because of its beneficial effects on skin it is a component of many cosmetic products.

8.11.3 Stearic acid

Stearic acid is an 18-carbon chain saturated fatty acid. It is commonly known as octadecanoic acid. Chemical formula of stearic acid is $C_{17}H_{35}CO_2H$. Stearic acid is more abundantly found in animal fates as compared to vegetable fat.

Molecular weight	284.5g/mol
Density	0.870 g/ml
Solubility	Insoluble in water, soluble in ethanol, acetone
Melting Point	68.8℃



Uses:

1. Stearic acid is used in production of soap, detergent and other cosmetics.
2. It is utilized as surfactant and emulsifying agent.
3. It is used in food packaging.
4. It is also an ingredient of some pesticides.
5. Utilized for synthesis of other chemicals.

8.12 SUMMARY

1. Carbohydrates are one of the most important biomolecules and one of the three major nutrients (source of energy) along with proteins and lipids.
2. Carbohydrates can be aldoses or ketoses depending upon the presence or aldehyde or ketone group.
3. Carbohydrates are classified as monosaccharides, oligosaccharides and polysaccharides depending upon number of sugar molecules present.
4. Disaccharide sugars are generally sweet sugars.
5. Polysaccharides can be structural or storage polysaccharides.
6. Polysaccharides made up of similar repeating units are called as homopolysaccharides where as polysaccharides made up of different units are called as heteropolysaccharides.
7. Due to presence of asymmetric carbon atom monosaccharides exhibit the property of optical activity.
8. Sugar can be dextrorotatory or levorotatory depending upon whether the plane polarized light when passed through sugar solution is rotated towards right or left.
9. Lipids are major class of biomolecules and main structural component of cell membrane.
10. Lipids are made up of fatty acids and glycerol.

11. Lipids are classified as simple, compound and derived lipids based upon their structural component.
12. Simple lipids are represented by fats and oil.
13. Compound lipids in addition to basic components of lipid molecules contain additional groups such as phosphorous, sulphur, carbohydrate or protein.
14. Lipid containing protein moieties attached to them are called as lipoproteins and lipids containing sugar molecules attached are called as glycolipids.
15. Both glycolipids and lipoprotein are commonly found to be present in lipid bilayer structure of cell membranes.
16. Lipids are also classifies as saturated and unsaturated, Saturated fatty acids lipids possess only single bonds between C atoms whereas unsaturated fatty acids contains one or more double bond between C atoms.

8.13 GLOSSARY

- **Anti-inflammatory:** substance that reduces inflammation (redness, swelling, and pain) in the body
- **Chitin :**a fibrous substance consisting of polysaccharides, which is the major constituent in the exoskeleton of arthropods and the cell walls of fungi
- **Emulsifying agent:** An emulsifying agent (emulsifier) is a surface-active ingredient which adsorbs at the newly formed oil–water interface during emulsion preparation
- **Glycosidic bind :** A glycosidic bond or glycosidic linkage is a type of covalent bond that joins a carbohydrate (sugar) molecule to another group, which may or may not be another carbohydrate.
- M**utarotation:** Mutarotation is a change in the optical rotation of a solution
- **Pectin:** Pectin is a structural acidic heteropolysaccharide contained in the primary and middle lamella and cell walls of terrestrial plants.
- **Rancidity:** Rancidity, condition produced by aerial oxidation of unsaturated fat present in foods and other products, marked by unpleasant odour or flavour
- **Stereoisomerism:** In stereochemistry, stereoisomerism, or spatial isomerism, is a form of isomerism in which molecules have the same molecular formula and sequence of bonded atoms, but differ in the three-dimensional orientations of their atoms in space.
- **Xylan:** It is the polysaccharide accounting for 25%–35% of the dry biomass of woody tissues of dicots and lignified tissues of monocots, and comprises up to 50% weight in some grasses and cereal grain tissues.

8.14 SELF ASSESSMENT QUESTION

8.14.1 Multiple choice questions

1. Which of the following is a pentose sugar
(a) Erythrose (b) Ribose
(c) Glucose (d) Mannose

2. Select the correct combination of pentose
(a) Erythroses and fructose (b) Glucose and Erythrose
(c) Fructose and glucose (d) Ribulose and Arabinose

3. The polysaccharide present in cell wall of fungi is
(a) Glycogen (b) Starch
(c) Chitin (d) Keratin

4. Simple lipids are made up of
(a) Fatty acids and aldehydes (b) Fatty acids and ketones
(c) Fatty acids and alcohols (d) Fatty acids and amino

5. The common source of oleic acid is
(a) Olive (b) Palm oil
(c) Sunflower oil (d) All of the above

6. Stearic acid is commonly known as
(a) Pentomic acid (b) Heptonic acid
(c) Octadecanoic acid (d) Hexadecenoic acid

7. Which of the following is example of unsaturated fatty acid
(a) Lauric acid (b) Myristic acid
(c) stearic acid (d) Oleic acid

8. Heparin and hyaluronic acid belongs to which of the following class of carbohydrate
(a) Oligosaccharides (b) Monosaccharide
(c) Homopolysaccharides (d) Heteropolysaccharide

8.14.1 Answer Key: 1-(b) 2-(a) 3-(c) 4-(c) 5-(d) 6-(c) 7-(d) 8-(c).

8.14.2. State whether the following statements are true or false

1. Carbohydrates are polyhydroxy aldehydes or polyhydroxy ketones.

2. Monosaccharides are amorphous and colorless.
3. Monosaccharides are water soluble.
4. Glycogen is a common polysaccharide found in plants.
5. A compound having asymmetric carbon atom is optically active.
6. Concentration of oxygen in lipids is low as compared to carbohydrates.
7. Presence of wax on plant surface provides protection against parasites.
8. Oleic acid generally occurs free in nature.
9. Oleic acid undergoes reaction of carboxylic acid.
10. Unsaturated fatty acids contain only single bonds between C atoms.

8.14.2 Answer Key: 1-True, 2-False, 3-True, 4-False, 5-True, 6-True, 7-True, 8-False, 9-True, 10-False.

8.14.3. Fill up the following blanks

1. Carbohydrates are synthesized in plants by the process of _____.
2. General empiral formula of carbohydrates is _____.
3. _____ is used in production of soap.
4. Monosaccharides exist as _____ at room temperature.
5. Carbohydrates combine with lipids to form _____.
6. _____ are mirror image of one another.

8.14.3 Answer Key: 1. Photosynthesis, 2.Cn (H_2O) 3.Stearic acid 4.Solids 5.Glycolipids 6.Enantiomer

8.14.4. Very short answer type questions

1. Define carbohydrates.
2. Differentiate between aldoses and ketoses.
3. How dose homopolysaccharides differ from heteropolysaccharides?
4. Mention the function of presence of wax on plant surface?
5. What are derived lipids?
6. Define stereo isomers?
7. Inspite of high molecular weight monosaccharides are extremely water soluble why?
8. Giving example differentiate between disaccharides and tri-saccharides?
9. How are hemiacetals produced?
10. What is asymmetric carbon atom?
11. What are epimers?

8.15 REFERENCES

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- J L Jain. A Textbook of Biochemistry

8.16 SUGGESTED READINGS

- Lehinger. Biochemistry
- Styrer. Biochemistry.
- Satyanarayan. A Textbook of Biochemistry

8.17 TERMINAL QUESTIONS

8.17.1. Short answer type questions

1. With examples describe about Monosaccharides?
2. How dose fats (oil) Differ from wax?
3. Giving suitable example classify compound lipids?
4. Describe about saturated and unsaturated fatty acids?
5. Briefly describe about structural polysaccharides?
6. Classify monosaccharides based upon number of carbon atom?
7. Tabulate the constituent components, structure and properties of common disaccharides?
8. Classify polysaccharides?
9. Giving examples with structure explain about hexoses?
10. Mention the uses with structure of linolenic acid?

8.17.2 Long answer type questions

1. Classify carbohydrates with examples?
2. Mention about the proportion and uses and structure of following fatty acids
   (a) Oleic acid
   (b) Stearic acid
3. Classify lipids giving suitable examples?
4. Enlist the functions of lipids?
5. What are the functions of Carbohydrates?

UNIT-9-AMINO ACIDS, PROTEINS, ENZYMES AND CO-ENZYMES

Contents:

9.1 OBJECTIVES

- Understand the organization of proteins and identify their classification.
- Understand the process involved in protein synthesis.
- Comprehend concept of enzyme and coenzyme and enzyme classification.
- Analyze enzyme kinetics and different types of enzyme inhibition.

9.2 INTRODUCTION

Proteins are a major class of biomolecules present in living organisms. Proteins are required by every living organism for their growth development and also for metabolic activities. Proteins are made up of amino acids and classified based upon their structure, function and composition. In every living organism, proteins are formed by a process known as protein synthesis or translation. Enzymes are another important biomolecule which are also known as biocatalyst. They catalyze the biochemical reactions occurring inside living organisms. Enzymes increase the rate of reaction (conversion of substrates into product) by lowering activation energy required for the reaction. Enzymes are classified into six different classes based upon the nature and type of reaction they catalyze. Although enzymes increase the rate of reaction which they catalyze but they themselves are not altered or consumed during a chemical reaction. Lock and key hypothesis and induced fit model are the two-mechanism proposed for enzyme action. Several molecules known as inhibitor function to block or decrease the enzyme activity. Three different types of enzyme inhibition have been identified as competitive inhibition, non-competitive inhibition and uncompetitive inhibition. Enzymes are also proteins in nature. Hence, enzymes as well as proteins are required by living organisms for different metabolic pathways and physiological processes occurring inside living cells.

9.3 AMINO ACIDS AND AMIDES

Proteins are made up of amino acids. Amino acids are known as building blocks of proteins. Large number of amino acid are linked together to form a protein. There are hundreds of amino acid known to be present in nature out of only twenty amino acids are involved in the process of protein synthesis. Every amino acid is composed of common frame work in which C atom is bonded to H atom, amino acid, carboxylic acid and alkyl group (Fig. 9.1). It is this R group which varies in all amino acids to give unique structure and properties to amino acids (Fig. 9.2).

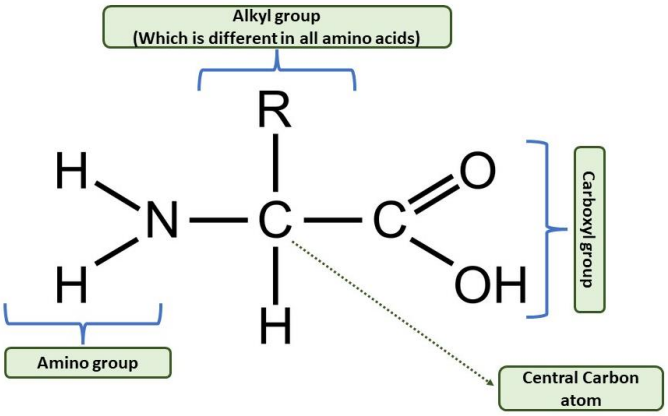


Figure 9.1: Basic structure of amino acid

Based upon nature of R group amino acids are classified in following groups:

Class of amino acids	Characteristic features	Examples
Simple amino acids	When no functional group is present	Glycine, Valine, Alanine
Hydroxy amino acids	Amino acid characterized by presence of OH group in side chain	Serine, Threonine
Sulphur containing amino acids	Presence of S atom in alkyl side chain	Cytosine, Methionine
Aromatic amino acids	Amino acids containing benzene ring in side chain	Tyrosine, Phenyl alanine
Heterocyclic amino acids	Amino acids in which side chain ring contain minimum one atom other than C atom	Tryptophan, Histidine
Amine containing amino acids	These are amino acid derivatives in which one of carboxyl group is transformed to amide group	Asparagine, Glutamine
Branched side chain amino acids	Amino acids in which aliphatic side chain is further branched	Leucine, Iso leucine, Valine

Essential and non essential amino acids

Amino acids which can be synthesized by animals and need not necessarily be provided with diet are known as non essential amino acids eg: Alanine, Glycine, Proline, Tyrosine. Where as essential amino acids cannot be synthesized by animals and should be provided with the diet eg: Lysine, Tryptophan, Methionine and Valine.

Acidic, basic and imino amino acids

Amino acids which contain carboxyl group in the side chain (R group) are called as acidic amino acids.eg: Aspartic acid, glutamic acid. Amino acid which contain amino group in side chain is

called basic amino acids eg: Lysine, Arginine. Amino acids which contain a secondary amino acid is called as imino acid eg: Proline.

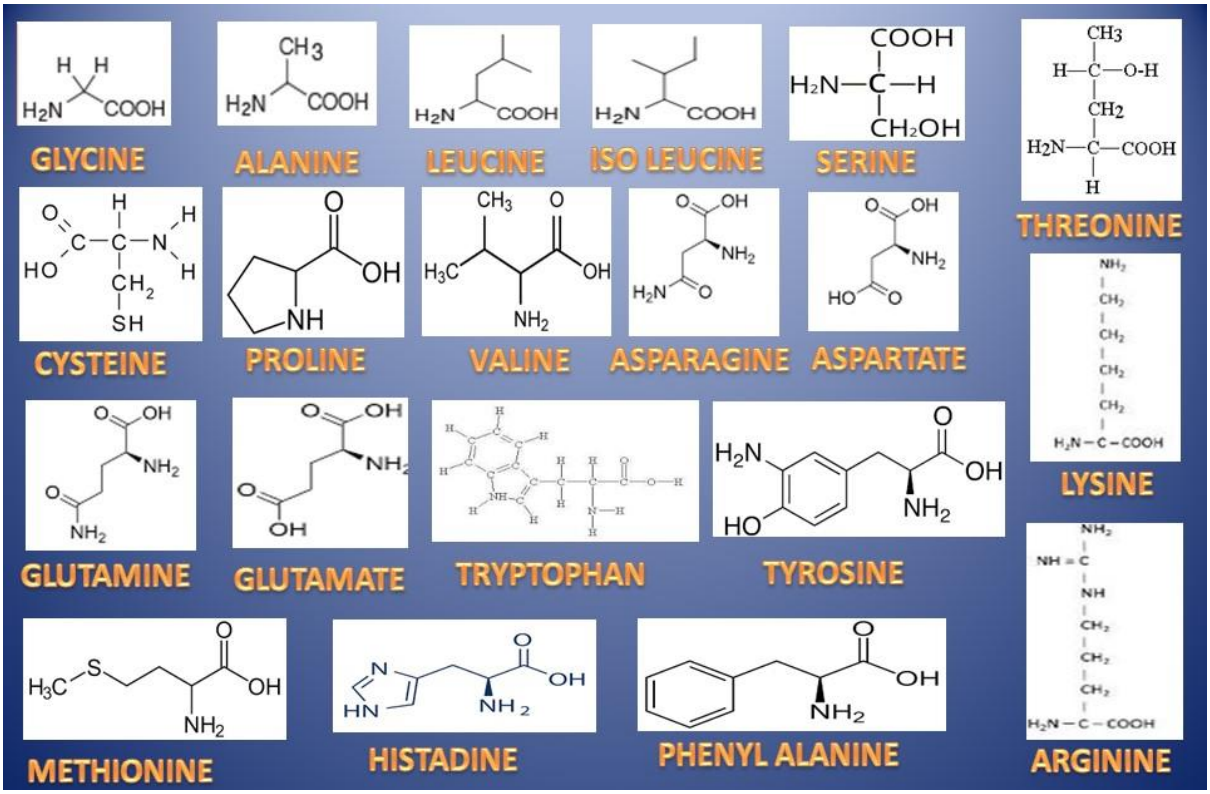


Figure 9.2: Structure of twenty amino acids involved in protein synthesis

AMIDES

Amides are the derivative of carboxylic acids. Amides are characterized by Carbonyl group bonded to N atom or group containing N. Similar reaction occurs when large number of amino acids join together to form a protein. In a protein chain every two amino acids are linked by peptide bond, a bond formed between COOH group of one amino acid and NH_2 of another amino acid resulting in formation of CONH bond with removal of water molecule.

9.3.1 General properties

A general pattern of synthesis of amino acid involves formation of amino acids from α-keto acids through a process known as transamination.

1. Amino acids are colorless and crystalline solids in nature.
2. Amino acids are water soluble and sparingly soluble in organic solvent with length of side chain (alkyl group) having a crucial impact on solubility of amino acids. Solubility of amino acids also depends upon pH and temperature of solvent. Amino acid tyrosine is soluble in hot water.
3. Amino acids have considerably high melting points ranging upto 200-300^{o} C.

4. Amino acids are stereospecific due to presence of chiral (asymmetric) carbon atom. Two stereoisomeric forms of amino acids are known as levorotatory (L-form) and dextrorotatory (D-form). Amino acids alanine, glutamine, isoleucine are dextrorotatory in nature where as amino acids leucine, phenylalanine and tryptophan are levorotatory.
5. Amino acids are amphoteric in nature i.e. they can act as acid and base.
6. Amino acids show chemical reactions of carboxylic, amino and alkyl group.
7. When heated to very high temperatures amino acids get decomposed.

9.3.2 Classification

Proteins exhibit large amount of diversity in their structure, composition and function. Hence it is difficult to classify them on one parameter. Hence proteins are classified based upon their structure, function, shape and nutritive value.

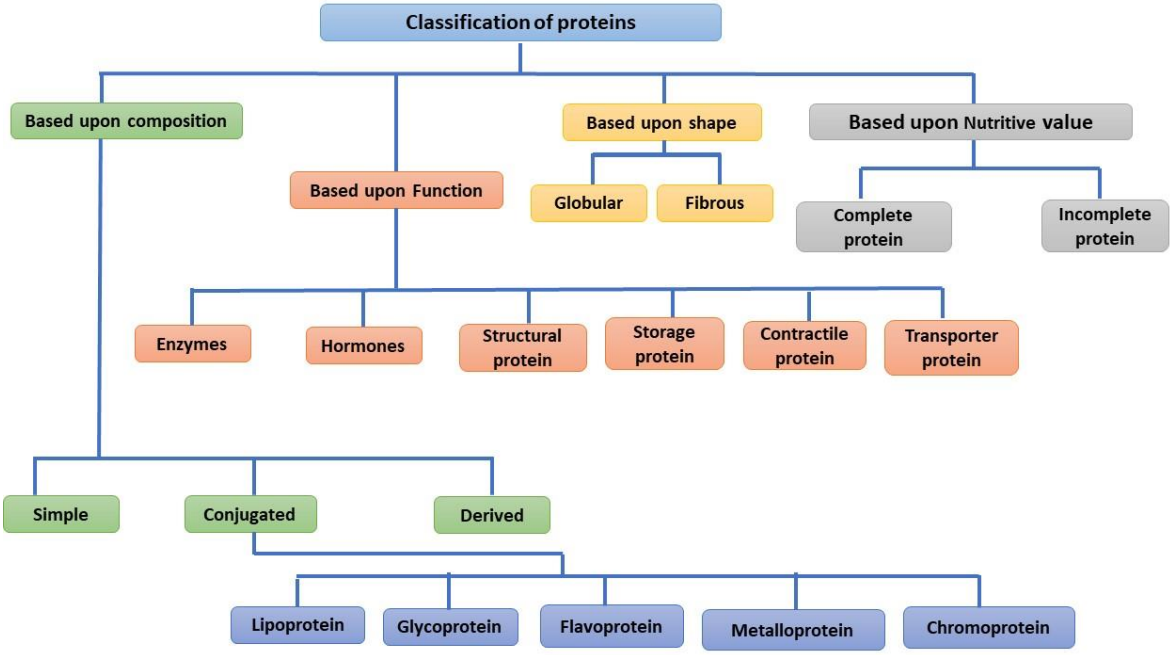


Figure 9.3: Classification of proteins

9.3.2.1 Classification based upon chemical composition of protein:

1. Simple protein:-Simple proteins are long chains of polypeptide made up of large number of amino acids.

Some of the common simple proteins include:

Simpleprotein	Characteristic feature	Examples
Albumin	• Soluble in water, dilute acids and alkali.	Ovalbumin, serum

	• Albumins get coagulated upon heating. • Most common source of albumin protein is egg.	albumin
Globulin	• Soluble in water. • Globulin get coagulated upon heating.	Myosin, globulin present in pulses.
Histones	• Histones are water soluble and basic in nature • Histones comprise very important class of protein as they are involved in packaging of DNA to form structure known as nucleosome. • Unlike albumin and globulin, histones are not coagulated by heat. • Histone proteins are absent in prokaryotes.	

2. Conjugated proteins: Proteins which contains molecules, groups, metal ions attached to polypeptide chains (made up of amino acids) are called as conjugated proteins. Attached group represents non protein part attached to the protein chain. The non protein part is known as prosthetic group.Generally conjugated proteins are globular shape and water soluble. Based upon the type and nature of prosthetic group conjugated protein are of following types:

Table 9.1: Different types of conjugated protein

	Conjugated protein	**Prosthetic group present**	**Examples**
1	Glycoprotein	Carbohydrate	Protein present in cell membrane, mucin (present in saliva)
2	Lipoprotein	Lipid	Membrane protein
3	Nucleoprotein	Nucleic acid	Protein present in ribosome and chromosome
4	Phosphoprotein	Phosphoric acid	Casein (milk protein), vitellin (egg protein in egg yolk)
5	Chromoprotein	Pigment	Haemoglobin, phytochrome (in plants)
6	Flavoprotein	Flavin adenine dinucleotide (FAD)	Proteins of electron transport chain.
7	Melalloprotein	Metal ion	Enzymes
		Zinc (Zn^{3+})	Alcohol dehydrogenase Carbonic anhydrase
		Nickel (Ni^{2+})	Urease
		Molybdenum (Mo)	Dinitrogenase
		Potassium (K^{+})	Pyruvate kinase Cytochrome
		Copper (Cu^{2+})	Oxidase

3. Derived proteins:

Derived proteins are the proteins obtained through denaturation or degradation of simple or conjugated protein. They are simple derivatives of large protein. Derived proteins are further classified as primary and secondary.

Primary derived proteins: Proteins derived either due to coagulative action of heat, acid or obtained due to initial hydrolysis of protein. Common example of coagulated protein are cooked food and boiled egg. Primary derived proteins have same molecular weight as native protein and also there is no change in peptide bond.

Secondary derived proteins: These proteins are obtained upon degradation of proteins which includes breaking of peptide bond. Proteases, peptones and peptides obtained upon degradative of large proteins are secondary derived proteins.

9.3.2.2 Globular and fibrous proteins: Globular protein are spherical in shape. Globular protein is generally functional proteins and involved in metabolic processes. They are water soluble. Globular proteins exhibit more sensitivity to temperature, pH as compared to fibrous proteins. Common examples of globular protein include insulin, pepsin, immunoglobulin, Fibrous protein, are rod like in structure, long and narrow. Fibrous protein is generally structural in function. They are less sensitive to pH and temperature and are insoluble in water. Common example of fibrous proteins includes myosin, fibrin, elastin and keratin.

9.3.2.3 Complete and Incomplete proteins: Proteins which contain all essential amino acids are called as complete proteins where as proteins which don not contain all essential amino acids are called as incomplete proteins. Animal meat is a good source of complete protein where as nuts, beans are common food source of incomplete protein.

9.3.2.4 Proteins are also classified into six different categories based upon their function.

1. Enzymes: Many proteins act as enzyme i.e., biocatalyst which increase the rate of biochemical reaction occurring inside living organism. Enzyme decreases the activation energy and increase rate of reaction which they catalyze. Enzymes are also required for various physiological and molecular processes such as digestion, DNA replication, etc.

2. Storage protein: Specific protein function as “storage proteins” both in plants as well as animal for growth e.g.: Seed protein, Ovalbumin in egg.

3. Structural protein: Proteins such as elastin, keratin, collagen is known as structural proteins as they function in formulation maintenance of structural components of living organism like hair, feathers, nails, horns, bones and other structural proteins are fibrous in nature and water insoluble.

4. Contractile proteins: Actin and myosin are two well known contractile proteins. Proteins which can contract (by utilizing ATPs energy) are known as contractile proteins.

5. Hormones: Hormones are regulatory molecules which control metabolic and physiological process occurring in plants and animals. Living organisms require hormones for their normal growth and development; several hormones are protein in nature which act as signaling messenger or regulatory proteins.

6. Transporter proteins: In every living cell transport of solutes and molecules occurs through cell membrane (either from cytoplasm to out of the cell or from extracellular matrix to inside the cell). Proteins are important structural component of cell membrane and many molecules are transported through these membrane proteins. Such membrane proteins are known as transporter proteins.

9.4 PROTEIN STRUCTURE

Proteins are organized into four different organizational levels known as primary, secondary, tertiary and quaternary.

1. Primary structure

Primary structure of a protein is polypeptide chain made of large number of amino acids. Amino acids present in polypeptide chain are linked to one another by formation of peptide bond between every two amino acids. Peptide bond formation involved linkage between carboxylic group of one amino acid and amine group of adjacent amino acid, in the process one molecule of water is eliminated resulting in formation of CO-NH bond (Fig. 9.4a). The polypeptide chain formation begins with formation of peptide bond between two amino acids resulting in formation of dipeptide, then a third amino acid is added resulting in formation of tripeptide. Large numbers of amino acids get linked to worm a polypeptide chain (Fig. 9.4b).

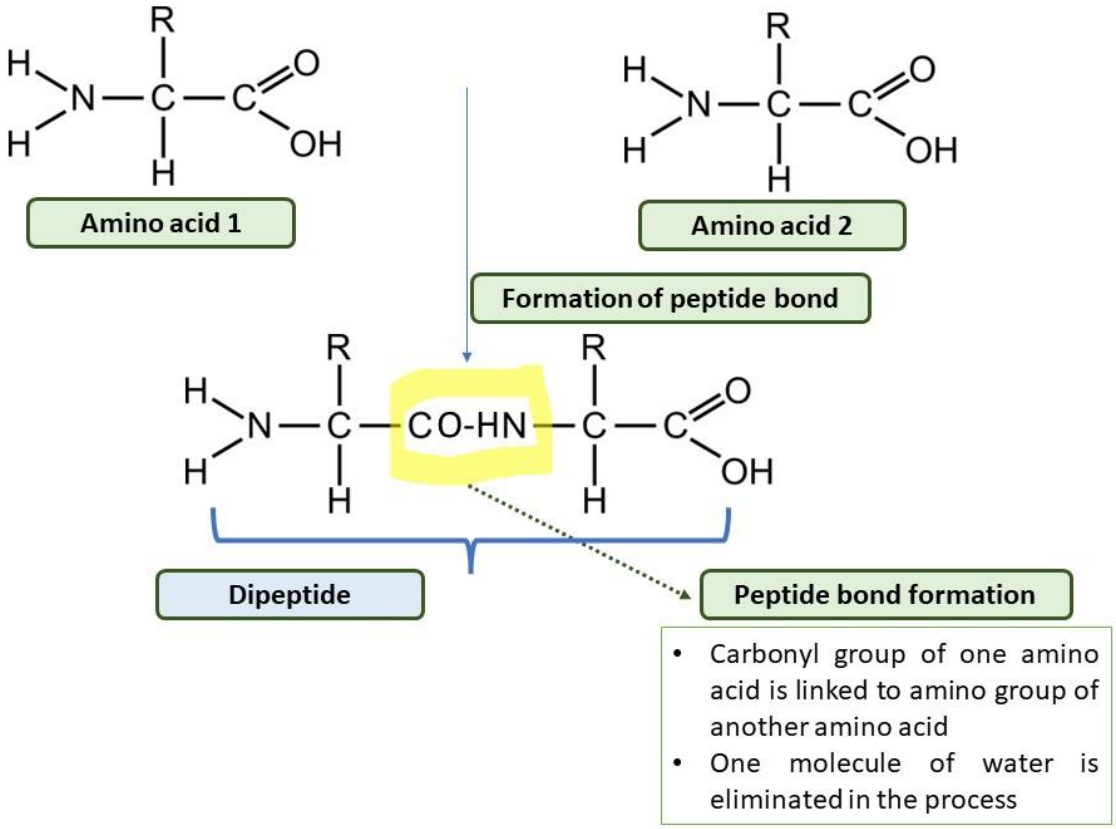


Fig. 9.4 (a): Peptide bond formation

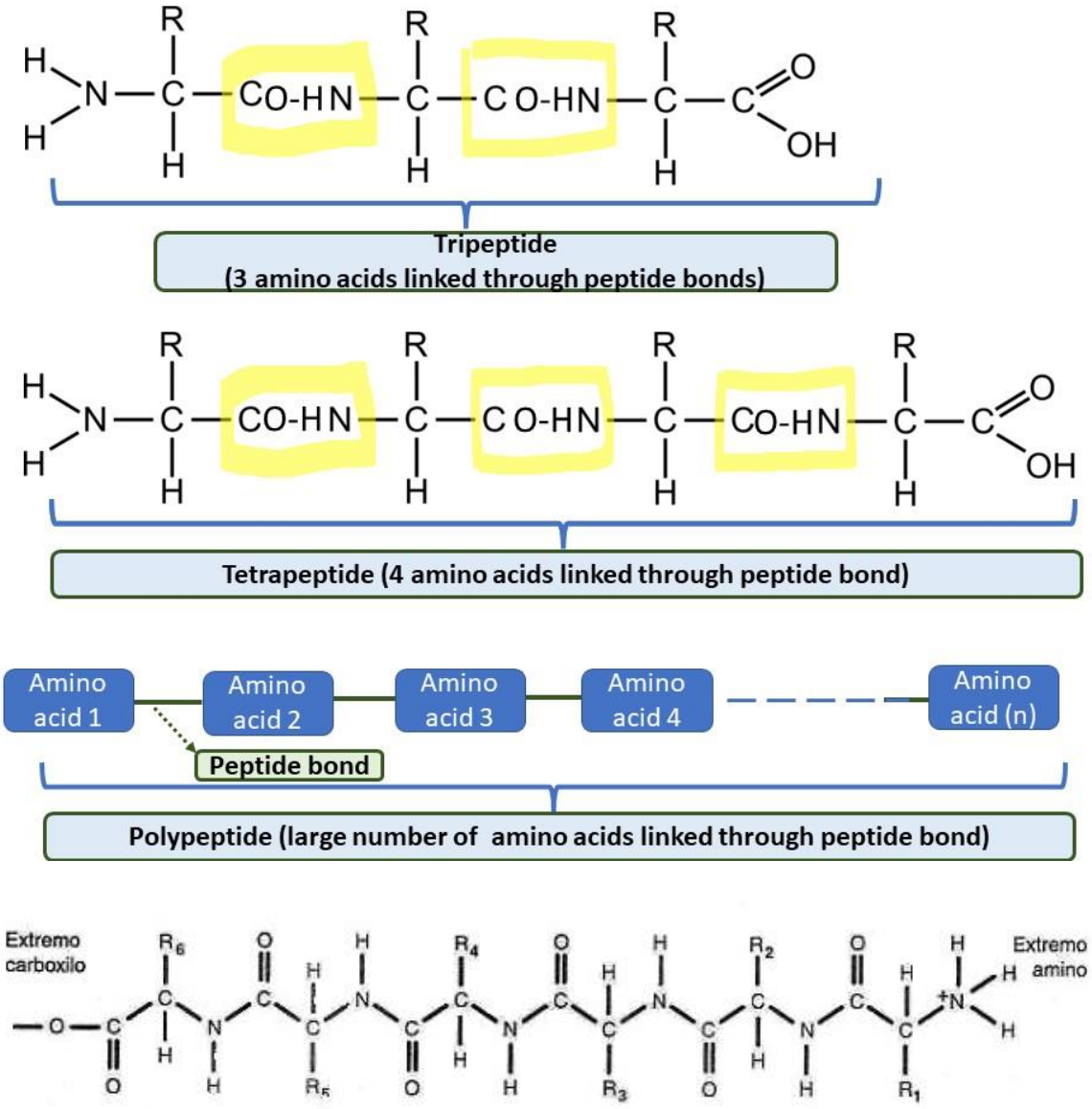


Figure 9. 4 (b): Structure of a polypeptide chain

2. Secondary structure of protein

Secondary structure of proteins is formed by folding of polypeptide chain in number of ways. Two most commonly known secondary structure of protein are α-helix and β-pleated.

α-helix is a rod (rigid in nature) like structure in which polypeptide chain is coiled like a spring (Figure. 9.5). Each turn of α-helix contains 3.6 amino acid residues. Intermolecular hydrogen bonding between –N–H and –C=O groups confers stability to α-helix structure of proteins. This intermolecular hydrogen bond formation occurs at every fourth amino acid present in the helix. α-helix structure common in both fibrous and globular proteins. Second type of secondary structure of protein is called as β-pleated. It involves hydrogen bonds formation between two or more polypeptide chains resulting in formation of a structure with sheet like appearance. H bond

occurs between –N–H group of one amino acid in polypeptide chain and –C=O of another amino acid in adjacent polypeptide chain. Secondary structure is common in fibrous proteins. β- pleated sheets are classified as parallel or anti-parallel (Figure. 9.6). In parallel arrangement all the polypeptide chains are running in same direction and in antiparallel structure the adjacent polypeptide chains in a β-pleated structure are oriented in opposite direction. Antiparallel structure favors maximum hydrogen bonding.

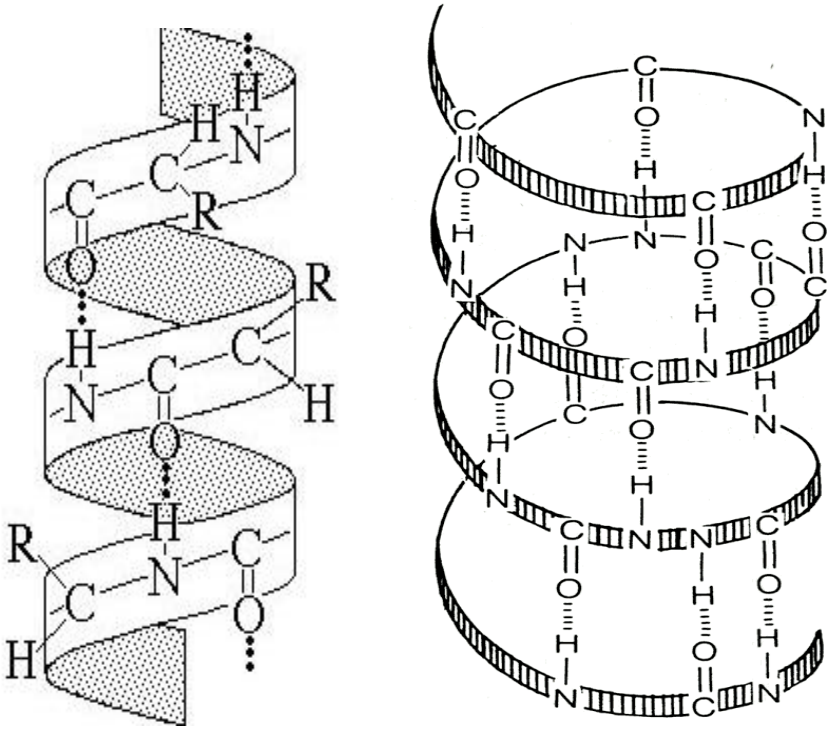


Figure. 9.5: Structure of α-helix secondary structure of protein

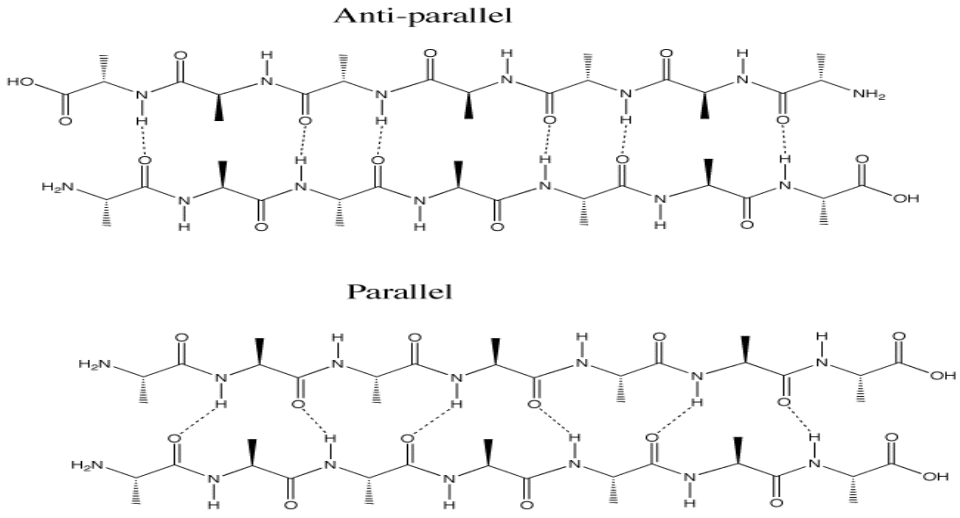


Fig. 9.6: Structure of β-pleated secondary structure of protein

Table 9.2 Differences between α-helix and β-pleated secondary structure of proteins

α-helix	β-pleated
Rod like (spirally coiled) appearance	Sheets like appearance
Formed through coiling of single polypeptide chain.	Formed by interaction of two or more polypeptide chains
Hydrogen bonds are formed with in the same polypeptide chain.	Formation of hydrogen bond occurs between different polypeptide chains
R groups are oriented outside of helix	R groups may be oriented towards inside or outside

3. Tertiary structure of protein

Tertiary structure of proteins is three-dimensional (3D) arrangement of polypeptide chains which include both α-helix and β-pleated sheets (Figure. 9.7). Tertiary structure of protein is formed by interaction between side chains or between sidechains and polypeptide chain. Tertiary structure is a complex structure (compared to secondary structure) due to protein folding. The biological activity and properties of each protein depends upon this protein folding. Tertiary structure of proteins is stabilized by hydrogen bonding, disulphide bonds, electrostatic interaction and hydrophobic interactions.

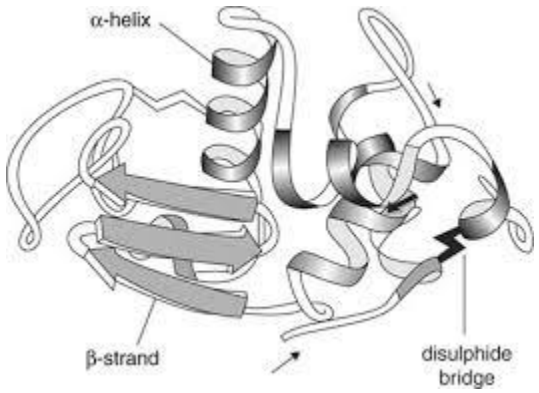


Figure. 9.7: Structure of tertiary structure of protein

4. Quaternary structure of protein

Quaternary proteins comprise of more than one polypeptide chain. Each polypeptide chain represents a subunit. Similar to tertiary proteins, quaternary protein structure is also stabilized by hydrogen and disulphide bonds, electrostatic interaction and hydrophobic interactions.

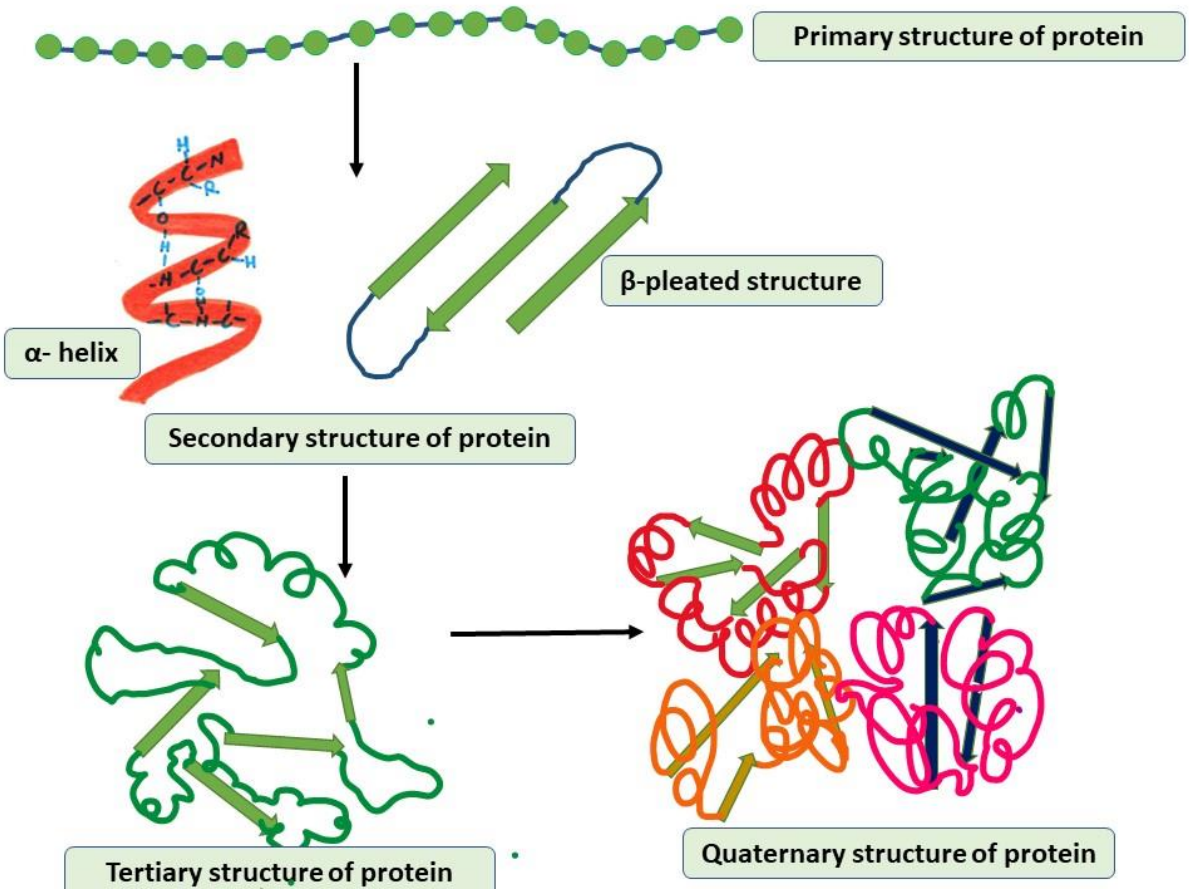
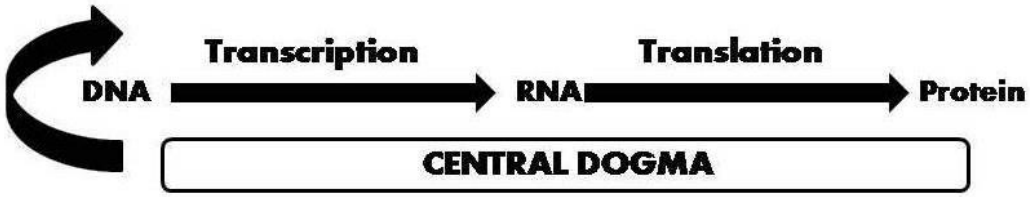


Fig. 9.8: Different level of structure of protein

9.5 Protein synthesis

Proteins are made up of large number of amino acids liked to one other by peptide bond.Protein is an important nutrient required by living organism for growth and maintenance. Proteins are synthesized in living cells by a process called as translation or protein synthesis. Protein synthesis is the last step of central dogma (a process occurring in living cells), in central dogma RNA is synthesized from DNA by a process known as transcription and protein are synthesized with the help of m-RNA by the process of translation.



The three main components required for the process of protein synthesis are:

1. m-RNA

2. Ribosome
3. tRNA

1. m-RNA

Messenger RNA (mRNA) is a type of RNA formed by the process of transcription from DNA. mRNA is single stranded and comprises 5% of total RNA found in the cell. mRNA is transported from nucleus to cytoplasm where it combines with ribosomes for the process of protein synthesis.

2. Ribosome

Ribosomes are the sites of protein synthesis in both prokaryotic and eukaryotic cells. Ribososmes are designated according to their rates of sedimentation.

- 70S for bacterial ribosome (have two subunits)
- 80S for eukaryotic ribosome (have two subunits)

The 'S' refers to the Svedberg unit. The smaller and larger subunits of ribosomes decodes mRNA and linked amino acids together through peptide bond respectively.

Prokaryotic ribosomes (70S):

The smaller subunit (30S) consists of 16S rRNA and 21 proteins, the larger subunit (50S) is composed of the 23S and 5S rRNA and 34 proteins.

Eukaryotic ribosomes (80S):

The smaller subunit (40S) of eukaryotic ribosomes is composed of 18S rRNA and 30 proteins (approx.) and larger subunit (60S) contains the 28S, 5.8S and 5S rRNAs and about 45 proteins.

3. tRNA

tRNA or transfer RNA is the second most common RNA in the cell. t-RNA has molecular weight 25,000 – 30,000, comprises of 73-93 nucleotides and is about 10-20% of total RNA present in the cell. The function of tRNA is to carry amino acids to mRNA during protein synthesis. Each amino acid is carried by a specific tRNA.

Process of protein synthesis (Translation)

1. Activation of amino acids: The process of protein synthesis begins with activation of inactive amino acids present in cytoplasm. Each amino acid is catalyzed by enzyme amino acyl +RNA Synthetase resulting in amino acyl adenylate formation. A high energy acyl bond formation occurs between carboxylic group of amino acid and α- phosphate of ATP. The other two phosphates of ATP are removed as inorganic pyrophosphates (PPi). Out of L- amino acids and D- amino acids only L- amino acids are involved in the process of protein synthesis.

$$\textit{Amino acid} + \textit{ATP} + \textit{Amino acyl} + \textit{RNA synthetase}$$

$$\downarrow$$

$$\textit{Amino acyl adenylate Synthatase} + \textit{Inorganic pyro phosphate}\ (\textit{PPi})$$

2. Transfer of amino acid to t-RNA: Activated amino acids are transferred to t-RNA, process known as charging of t-RNA. There are about 100 different types of t-RNA found in cytoplasm. Each activated amino acid is transferred to its specific t-RNA by formation of an ester bond between carboxylic group of amino acid and 3' –OH group of terminal adenosine of tRNA.

3. Initiation of synthesis: Shine Dalgarno (SD) sequence is a ribosomal binding site found only in prokaryotic (absent in eukaryotes) mRNA. It is found to be located at 8-10 base pair upstream of start codon (AUG). The sequence helps in recruitment of smaller ribosomal subunit to mRNA after which protein synthesis starts.

For initiation of protein synthesis different initiation factors (IF) are required. In Prokaryotes IF-1 and IF-2 are required for binding of tRNA to 30S ribosomal subunit. IF-2 is also needed for binding of guanosine triphosphate (GTP). Initiation factors in eukaryotes are named as eIF-2, eIF-2', eIF-2a, eIF2a2, eIF-2a3 and eIF-3. eIF- 2 forms a complex between met-tRNA and GTP. This complex binds to 40S ribosomal subunit to form 40S initiation complex. eIF-2' helps in binding of met- tRNA to 40S subunit in absence of GTP. This is an AUG dependent process. eIF-2a, eIF2a2, eIF-2a3 (accessory factors) are needed for binding of met- tRNA along with GTP.

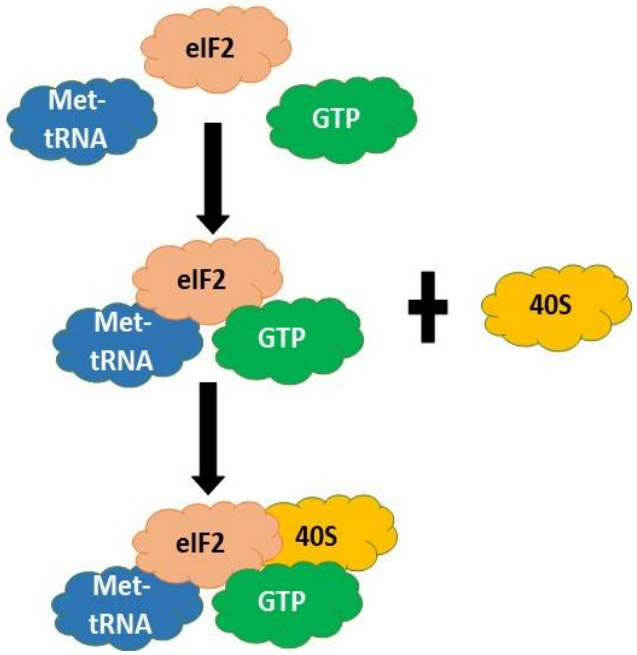


Figure 9.9: Components required for formation of initiation complex in eukaryotes

Formylation of Methionine: After the formation of initiation complex formylation of methionine occurs. In eukaryotes starting N-terminal amino acid is methionine, which binds to methionine to form methionyl – tRNA+met and in prokaryotes the starting methionine amino acid carries a formyl group hence called as N-formylmethionine which binds to In prokaryote initiation tRNA to form N- formyl methionyl – tRNA +met.

Formation of 30S initiation Complex

mRNA gets attached to 30S ribosomal subunit to form mRNA-30S complex. IF-3 is required for this process to occur. To mRNA-30S complex, fmet–tRNA is a added. IF-2 and IF-1 along with GTP are required.

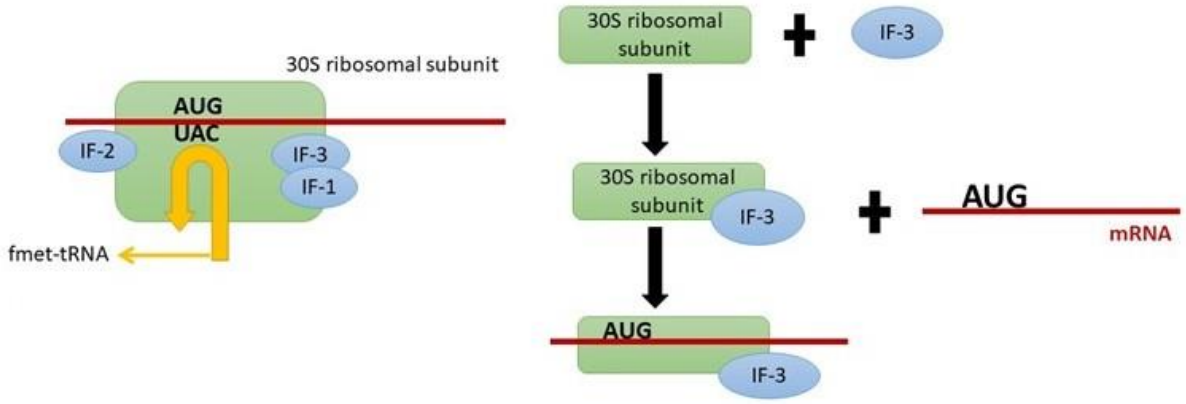


Figure 9.10: Formation of 30S initiation complex

Factor required for binding of fmet-tRNA to 30S complex

The mRNA contains AUG (initiation codon) and the tRNA which is attached first of all contains UAC anti codon. In eukaryotes initially Met–tRNA binds to 40S ribosomal subunit and then mRNA attaches with its AUG Codon. After tRNA gets attached to mRNA -30 subunit complex next is addition of 50S subunit of ribosome. 50S subunit of ribosome contains two binding sites in which two tRNA molecules can bind.

A site : It is called aminoacyl or acceptor site.

P site : It is called peptidyl or polymerisation site or donor site.

The initiator tRNA, fmet-tRNA always directly binds to p site. However, all other tRNA (which all bind after initiator tRNA bind to P site), first binds to A site and then they are translocated to P site. During attachment of 50S subunit GTP is converted to GDP+Pi and initiation factors IF-1 and IF-2 are related for recycling.

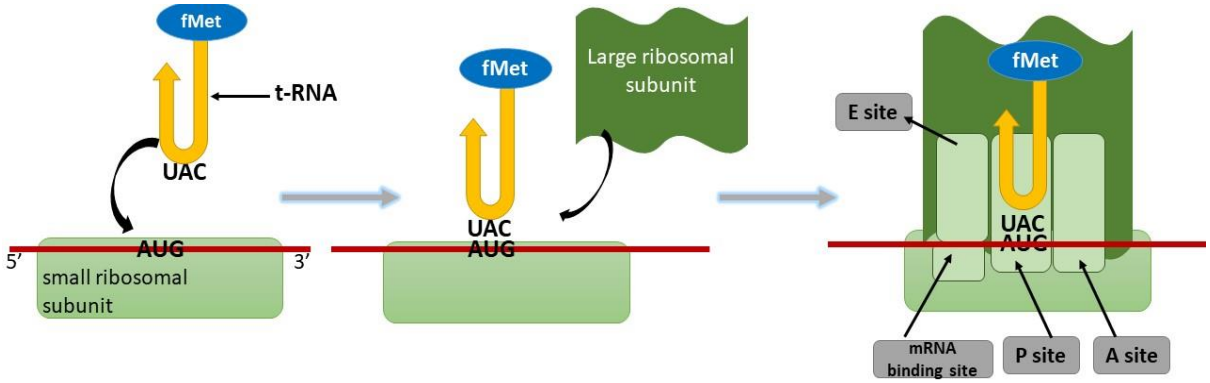


Figure 9.11 Summary of initiation of protein synthesis

4. Elongation of polypeptide chain:

Several elongation Factors (EF) are involved in elongation of polypeptide chain. EF-1 in eukaryote and EF-Tu along with EF-Ts in prokaryote functions to bring aa-tRNA to A site of ribosome. EF-2 in eukaryotes and EF-G in prokaryotes bring about translocation of aa-tRNA from A site to P site. Elongation factors EF-T4 and EF-T5 in prokaryotes are required for binding of amino acyl tRNA ribosome. EF-T4 also forms a complex with aa tRNA and GTP.Once the initiator tRNA containing initiation aa1 binds to P site and another tRNA with aa2 binds to A site. Now a peptide bond will be formed between the aa1 (amino acid present in P site) and aa2 (amino acid present in A site).

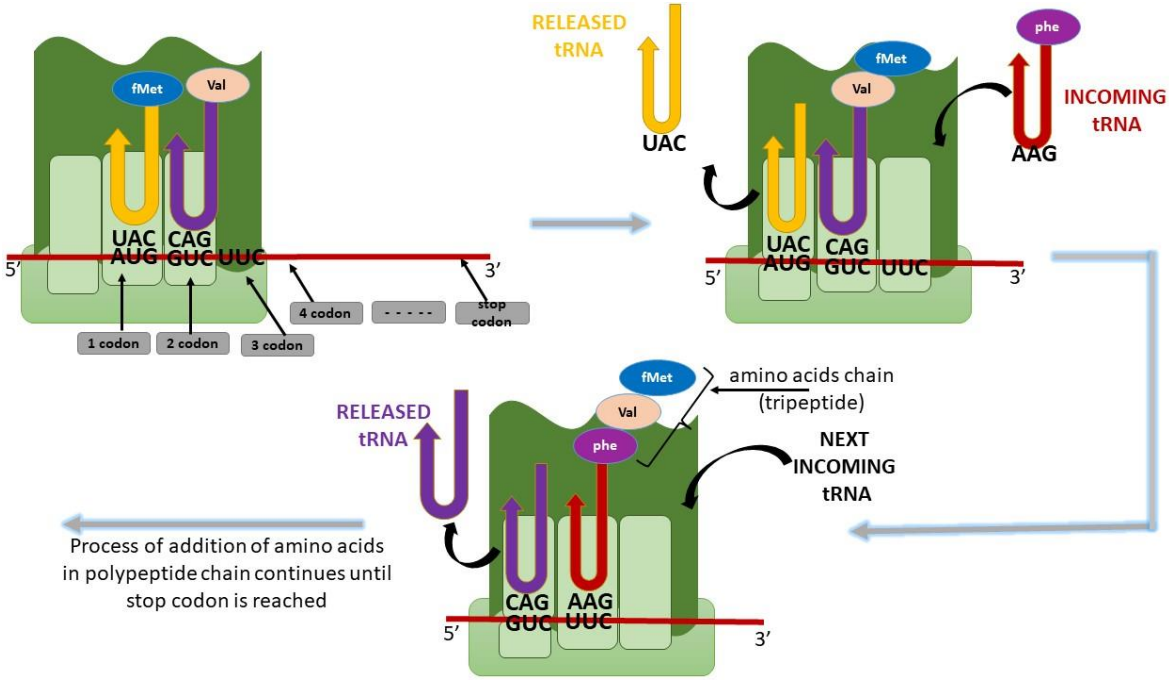


Figure 9.12: Summary of elongation of protein synthesis

After peptide bond formation t-RNA molecule of fmet–tRNA is released from P site the second t-RNA from A site is (with third amino acid) transferred to P site and a third t-RNA comes into A site. Now a second peptide bond will be formed between second amino acid and third amino acid. Again second t-RNA from P site is released and third t-RNA from A site will be translocated to P site and A site will become free for entry of next t-RNA molecule.In this way one by one t-RNA molecules will keep on entering through A site and then transferred to P site. Before each transfer process a peptide bond will be formed between amino acid attached to t-RNA in A site. By this process large number of amino acids will be linked to one another by formation of peptide bond resulting in polypeptide (protein) chain formation.

The t-RNA molecule to which the growing polypeptide chain is attached is called peptidyl tRNA. This remains bound to P site. The incoming tRNA molecule having amino acid molecule is called aminoacyl tRNA which binds to A site. For the above process to occur one by one tRNA will keep on entering via A site now there are so many tRNA available how a specific tRNA will be selected A site. This will occur according to the codon sequence in mRNA. The ribosome will keep on moving along mRNA in 5'-3' direction and for every codon present on mRNA, a tRNA with anticodon will be selected. This movement of ribosomes along mRNA is known as translocation.

5. Chain termination

The elongation of polypeptide chain continuous until a stop codon (UAA, UAG, UGA) is read on mRNA. Stop codons or termination codons does not code for any amino acid, hence when the ribosome moving along m RNA reaches any of three stop codons process of protein synthesis stops. Once the synthesis of polypeptide chain stops, the polypeptide chain still remains attached to tRNA molecule.

Now, specific release factors bring out hydrolysis of polypeptide chain at P site and all the components of protein synthesis assembly (linked for the process of protein synthesis) get separated and released. Ribosome gets dissociates into smaller and larger subunits. In prokaryotes there are three known release factors RF1, RF2 and RF3. Release factors RF1 and RF2 recognize stop codons and RF3 helps in stimulating binding and release of RF1 and RF2 from the ribosome. In eukaryotes there is only one release factor (RF) and this single release factor recognizes all the three stop codons (UAA, UAG and UGA).

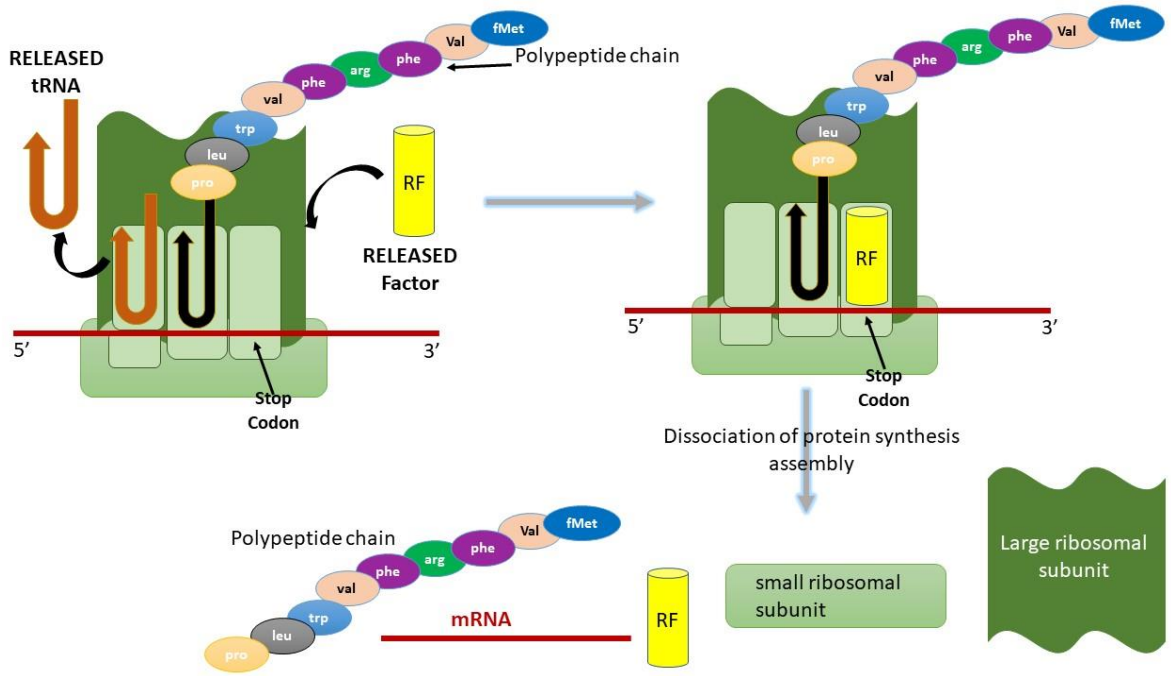


Figure 9.13: Summary of termination of protein synthesis

9.6 ENZYMES AND CO-ENZYMES

Metabolism of living organisms comprises of large number of chemical reactions occurring inside living cells. These reactions can be characterized as catabolic or anabolic. Reactions occurring in living organisms can also be characterized as exergonic (reactions in which energy is liberated) or endergonic (reactions for which energy is required). Several of these biochemical reactions require enzymes as catalyst. The term enzyme was coined by Friedrich W Kuhne in 1878. Enzymes are defined as biocatalysts which enhance the rate of reaction by lowering activation energy.

General properties of enzymes:

- Enzymes are protein in nature, which catalyze biochemical reactions.
- Enzymes are specific in their activity i.e, each type of enzyme catalyzes a particular type of reaction.
- Catalytic activity and specificity of enzymes is attributed to their tertiary or quaternary structure.
- All the enzymes possesses an active site where substrate molecule binds and formation of products is catalyzed.
- Enzymes themselves donot gets chemically altered during a reaction.
- Every enzyme has its own turnover number (i.e, number of substrate molecules converted into product per unit time)

- Enzymes only enhance the rate of reaction. They have no influence on equilibrium of reaction nor do they affect nature and type of product formed.

Cofactor and coenzymes

Many enzymes require cofactor for their catalytic activity. Cofactor is defined as non- protein part attached to enzyme which are essential foe enzyme activity. Cofactor molecules can be organic or inorganic. Common example of organic cofactor includehaeme, flavin and metal ions and their clusters are inorganic class of cofactors. Cofactors which are loosely associated with their enzyme are called as coenzymes and the cofactors which are tightly bound totheir enzyme are known as prosthetic group. Based upon presence or absence of prosthetic group enzymes are classified as holoenzyme or apoenzyme. Enzyme when associated with prosthetic group is called as holoenzyme (catalytically active) and when prosthetic group is not associated to enzyme, the enzyme is known as apoenzyme (catalytically inactive). Organic cofactors (generally having a complicated structure) are called as coenzymes. The main function o f coenzymes is to serve as intermediate carrier of electrons transferred or functional groups during a chemical reaction.

Table 9.3: List of some of coenzymes and their respective enzyme.

	Enzyme	**Coenzyme**
1	Dehydrogenases	Nicotinamide adenine dinucleotide (NAD)
2	Cytochromes	Flavin adenine dinucleotide (FAD) Flavin adenine mononucleotide (FMN)
3	Decarboxylases	Thiamine pyrophosphate
4	Glycogen phosphorylase	Pyroxidal phosphate
5	Pyruvate carboxylase	Biotin
6	Thymidylate synthase	Tetrahydrofolate

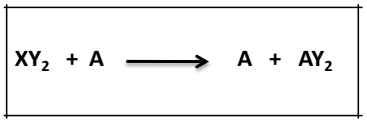
9.7 ENZYME CLASSIFICATION

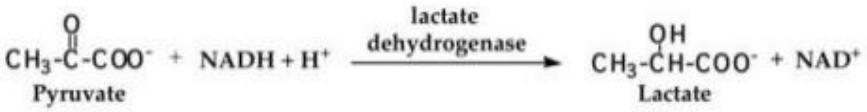
There are six different classes of enzyme based upon the type of biochemical reaction catalyzed by the respective enzyme. The six classes are as follows:

1. Oxidoreductases

Enzymes catalyzing oxidation – reduction reactions are known as oxidoreductases. C=O, CH-CH, CH-NH_2 and CH-OH are common substrates whose oxidation- reduction reactions are catalyzed by enzyme oxidoreductases.

General reaction :

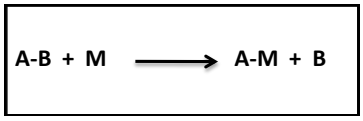


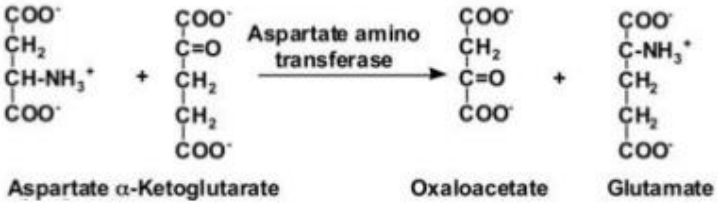


2. Transferases

Enzyme Transferase bring out (catalyze) transfer of one functional group from a compound to another compound.

General reaction:

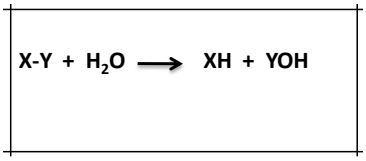


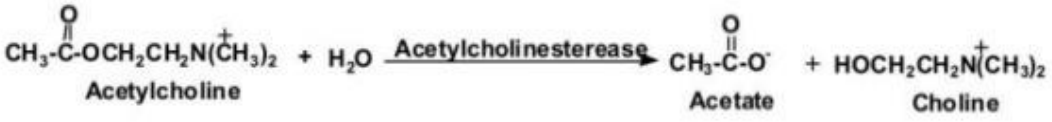


3. Hydrolases

Hydrolases are the enzymes catalyzing hydrolytic cleavage of C-O, C-C, C-N, P-N and other bonds. Hydrolytic cleavage means that these enzymes add a molecule of water across the bond which these enzymes break.

General reaction:

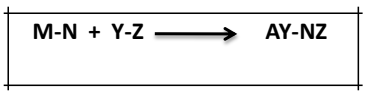


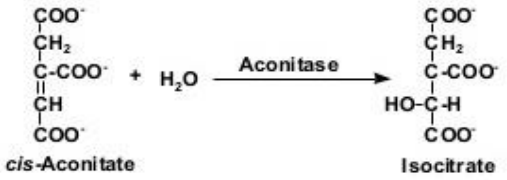


4. Lyases

Enzymes belonging to the class lyases catalyze cleavage (break) of bonds such as C-C, C-N, C-O, C-S C-halogen (F, Cl, Br, I) by any mechanism other than hydrolysis. Such bond breakage results in formation of double bond or formation of ring structure.

General reaction:

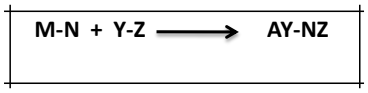


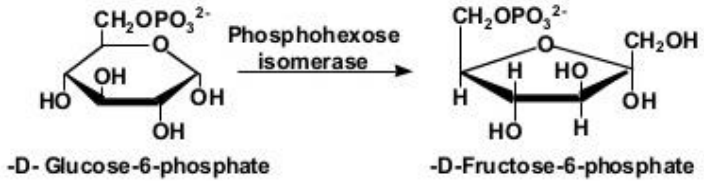


5. Isomerases

Isomerases are the enzymes which catalyze structural or geometric changes in a molecule. This occurs by causing intramolecular arrangement of atoms or groups. Depending upon the isomerism present in molecules these enzymes are further divided into different subclasses such as racemerases, epimerases, cis-trams isomerases, tautomerases and mutases.

General reaction:





6. Ligases

Ligases catalyze joining of two molecules by expenditure of energy (ATP). The energy required by ligases is obtained by breaking of pyrophosphate bond in ADP or ATP.

General reaction:

9.7.1 Mode of action

Enzymes are known as biological catalyst which increases the rate of reaction by decreasing activation energy of the reaction (Figure 9.14).

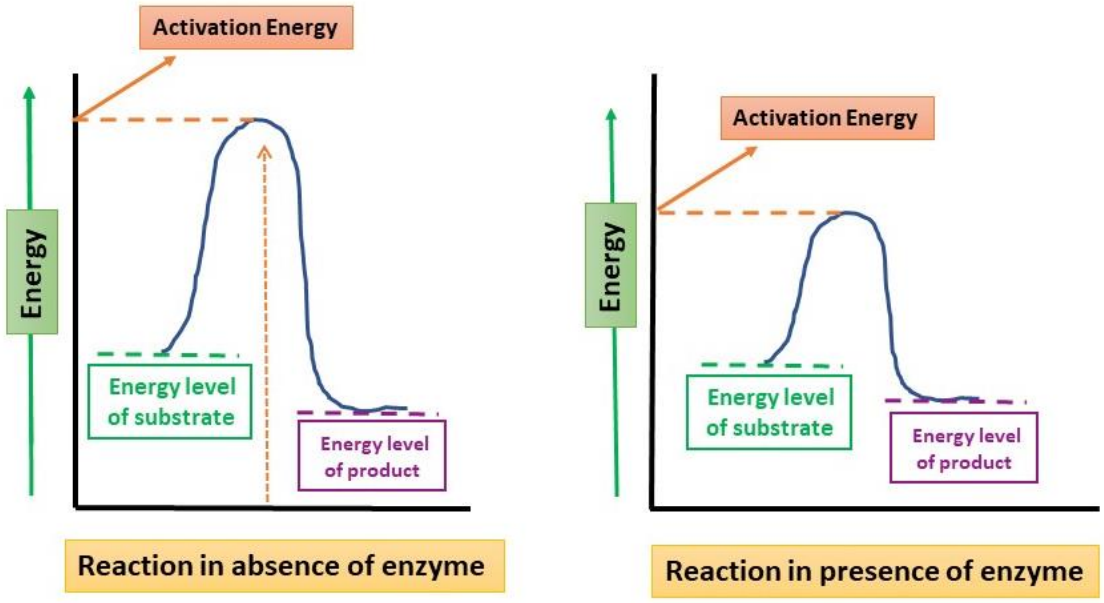


Figure 9.14: Effect on enzyme catalysis on activation energy

There are two proposed mechanism of how a substrate molecule binds to an enzyme (at active site) and how enzymatic reaction is catalyzed.

(A) Lock and key hypothesis

Lock and key mechanism of enzyme action was given by Emil Fisher in 1894. The mechanism is called as lock and key because as lock has a specific key which can accurately fit into the key hole (of lock) similarly only a specific substrate molecule can bind to active site present on an enzyme (Figure 9.15).

Silent features of the model:

1. Enzyme possesses preformed active site with a specific conformation.
2. The conformation of active site is complementary to the shape of substrate molecule.
3. Due to structural complementarity substrate molecule binds to active site of enzyme.

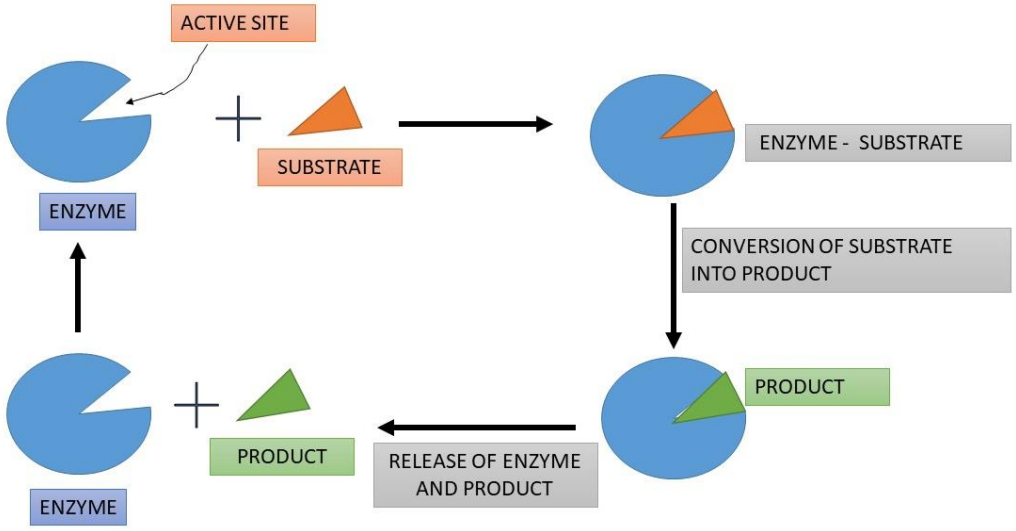


Figure 9.15: Lock and key mechanism of enzyme action

(B) Induced fit model

Second model for mechanism of enzyme action known as Induced fit model was proposed by Daniel Koshland (1958).

Silent features of the model:

1. According to this model (Figure 9.16) enzyme does not possess preformed active sites (complementary to substrate molecule).
2. When a substrate molecule approaches to an enzyme there is conformational modification in region of active site to facilitate binding of incoming substrate.
3. According to this model initial interaction between enzyme and substrate in comparatively weak but as the reaction proceeds alongwith conformational changes in structure of enzyme (active site modification) substrate enzyme binding strengthens.

Induce fit model of mechanism of enzyme action has several advantages. The mechanism supports action of enzyme on various (different) substrates which may differ in conformation. It is a much accepted mechanism as enzymes are not absolutely rigid, depending on different environmental conditions different interactions one promoted. Moreover in presence of an inhibitor induced fit mechanism of enzyme action enhances the chances of recognition of substrate molecule.

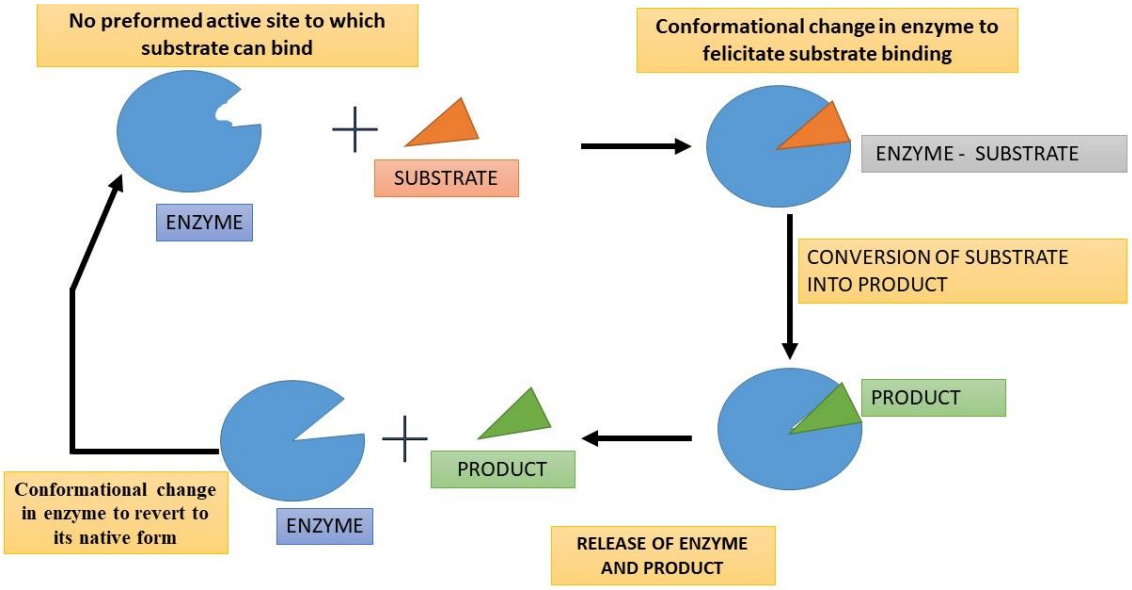


Figure 9.16: Induced fit model for mechanism of enzyme action

9.7.2 Kinetics

Enzyme kinetics refers to the study/analysis of rate of reactions catalyzed by enzymes. Enzyme kinetics involves measurement of rate of reaction and different factors affecting rate of reaction.Temperature, substrate concentration, pH, enzyme concentration, inhibitors and actuators are major factors affecting rate of enzyme catalyzed reaction. The most important aspect of enzyme kinetics is Michaelis-Menten equation. Leonor Michaelis-Menten derived a mathematical equation to calculate rate of enzyme catalyzed reaction depending upon on concentration of enzyme and substrate. According to their equation, Vmax represents maximum reaction rate (maximum velocity) and Km which is known as Michaelis constant is that concentration of substrate at which the rate of reaction is 50% of Vmax. Vmax is the point after which if the concentration of substrate is increased there will be no further increase in rate of reaction. This is because all the active sites of enzyme are occupied by substrate and there is no free active site to which a substrate can bind.

For reaction,

$$\boldsymbol{E + S \rightarrow ES \rightarrow E + P}$$

The Michaelis-Menten equation is represented as: $\boldsymbol{V = Vmax[S] / Km + [S]}$

9.7.3 ENZYME INHIBITION

Enzyme inhibition represents a process in which the action of enzyme is blocked (inhibited) by an inhibitor molecule. Inhibitors are chemical compounds / molecules which bind to enzyme to inhibit its activity. Binding of inhibitor to enzyme is either reversible or irreversible. In irreversible binding the inhibitor molecule permanently binds to the enzyme blocking its activity. However, in case of reversible binding the inhibitor molecule binds temporarily to the enzyme. It means that till the reversible inhibitor is bound to the enzyme its activity remains blocked when

the inhibitor is released the enzyme again becomes free to catalyze its respective reaction. A reversible inhibitor can bind to active site or to another site on the enzyme. In reversible inhibition inhibitor bind to enzyme through non covalent bonding where as in case of irreversible inhibition the inhibitor molecule binds to enzyme through covalent bond.

Types of enzyme inhibition

(1) Competitive inhibition

In competitive inhibition the inhibitor binds to active site of an enzyme. As the name indicates in competitive inhibition the inhibitor molecule competes with substrate molecule to bind to active site. The value of Km (Michaelis-Menten constant) increases whereas there is no change in Vmax. This is because in presence of excess substrate molecule reaction can still be completed. A competitive inhibitor is structurally / chemically similar to substrate because of which it is able to bind to active site. Once the inhibitor molecule binds to active site, binding of substrate to enzyme is prevented because active site is occupied by inhibitor and hence of enzyme is inhibited (Figure 9.17).

Examples of Competitive inhibitor

(1) Relenza

Relenza is a drug given to person infected with influenza virus. Relenza binds to active site of viral enzyme neuraminidase which eventually prevents release of virions from infected cells.

(2) Methotrexate

Enzyme dihydrofolate reductase (DHFR) catalyzes reduction of folate which is an important reaction in nucleotide metabolism. Drug methotrexate resembles folate and hence acts as competitive inhibitor.

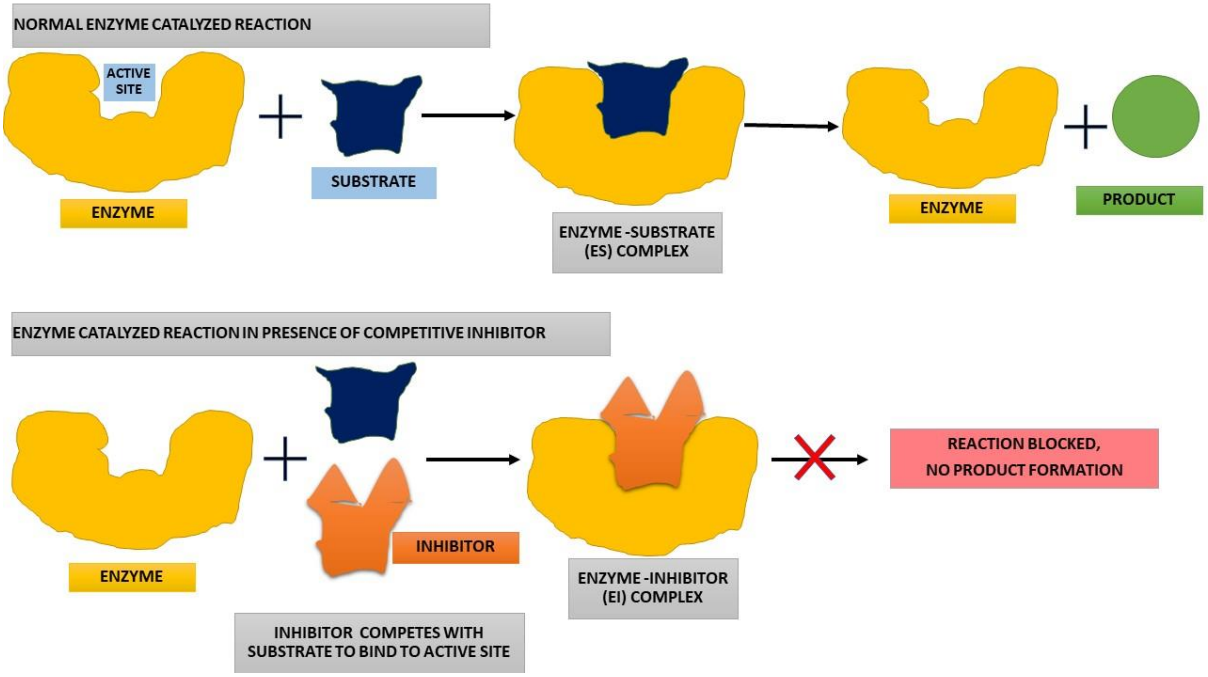


Figure 9.17: Summary of competitive inhibition

(2) Non-competitive inhibition

In non- competitive inhibition the inhibitor molecule binds to alternative site (other than active site) on enzyme. The value of Vmax decreases in non-competitive inhibition of enzyme whereas Km remains unchanged. As the name suggests in non-competitive inhibition there is no competition between the inhibitor molecule and substrate to bind to enzyme. This is because substrate binds to active site and non-competitive inhibitor binds to site other than active site. But once the inhibitor binds to enzyme there is conformational change in enzyme which restricts enzyme substrate binding hence resulting in enzyme inhibition.The site on enzyme to which non-competitive inhibitor binds is called as allosteric site. The degree of non-competitive inhibition cannot be reversed by increasing the concentration of substrate (Figure 9.18).

Cyanide is common example of Non-competitive inhibitor. Cyanide binds to allosteric site on enzyme cytochrome oxidase. Cytochrome oxidase is part of electron transport chain. When inhibited by cyanide the enzyme is note able to pose electron to final acceptor which stops ATP production.

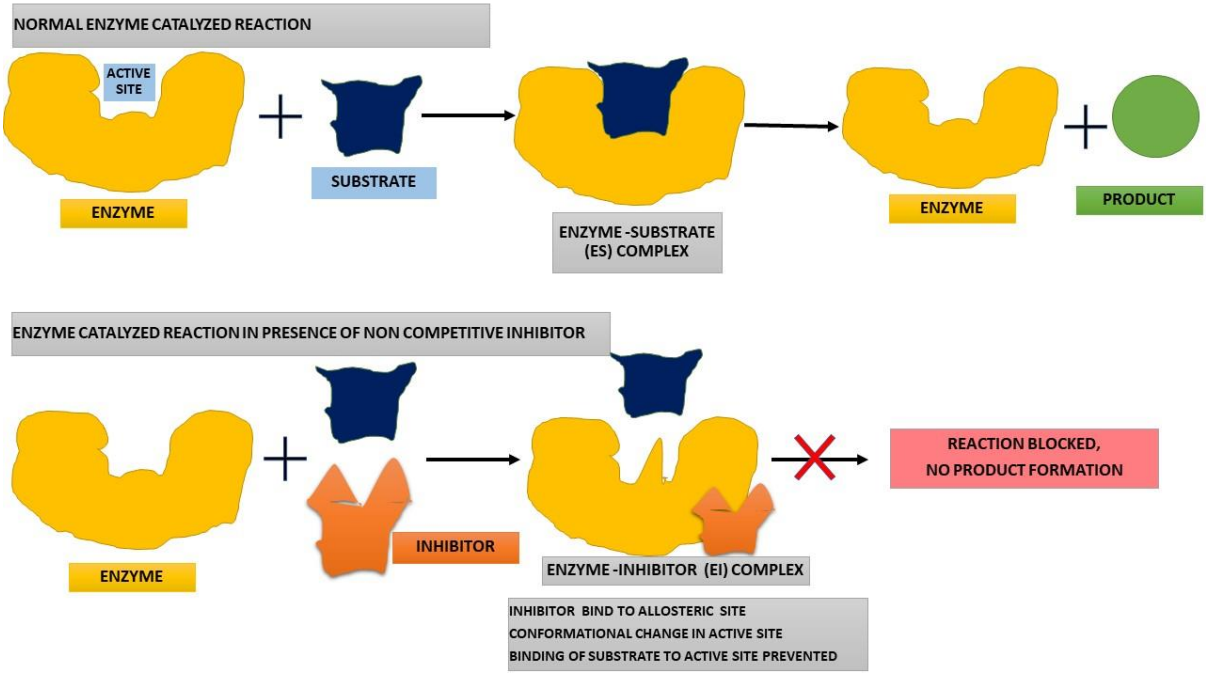


Figure 9.18.: Summary of non - competitive inhibition

Uncompetitive inhibition

In uncompetitive inhibition the inhibitor does no binds to free enzyme but binds to Enzyme Substrate Complex. As a result of binding of inhibitor to ES complex, conversion of substrate into products is blocked due to formation of inactive ESI (Enzyme-Substrate Inhibitor) complex. The value of Vmax is reduced during uncompetitive inhibition. Uncompetitive inhibition is rare in one substrate reaction and more common in bi-substrate reaction.

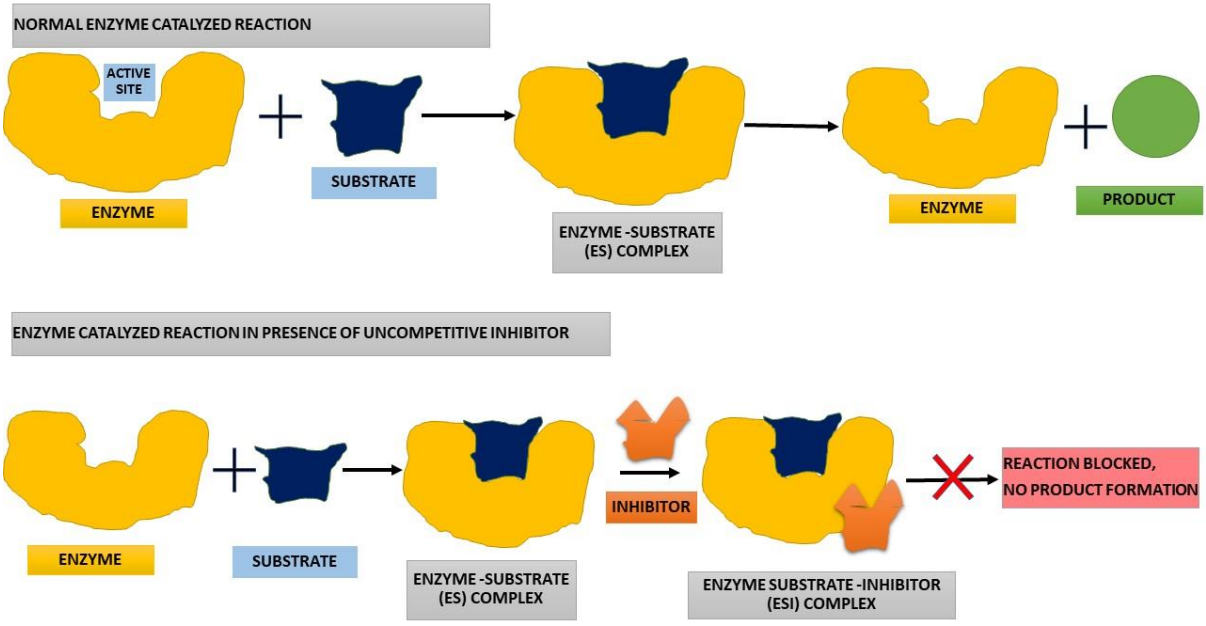


Figure 9.19: Summary of uncompetitive inhibition

9.8 SUMMARY

1. Proteins are major class of biomolecules made up of amino acids.
2. Proteins are classified as sample conjugated or derived based upon their composition.
3. Depending upon their shape proteins can be globular or fibrous.
4. Structural organization of protein includes primary, secondary, tertiary and quaternary structure of proteins.
5. The process of formation of proteins inside the cells is known as protein synthesis or translation.
6. Major components involved in the process of protein synthesis are mRNA, ribosomes and t-RNA.
7. The process of protein synthesis is divided into three parts initiation, elongation and termination.
8. Enzymes are bio catalysts which enhance the rate of reaction.
9. Enzyme catalyzes the reaction by lowering the activation energy.
10. Enzyme contains active sites to which substrate binds.
11. Substrates bound to enzyme are converted into products.
12. Number of substrates converted into products per unit time is known as turnovers number.
13. Cofactors are non-protein part attached to enzyme which are required for enzyme activity.
14. Enzymes are classified into six different classes based upon the type of reaction they catalyze.

15. Two mechanisms proposed for enzyme action are lock and key hypothesis and induced fit model.
16. According to lock and key hypothesis enzymes possess preformed active site to which substrate binds.
17. According to induced fit model enzymes do not contain preformed active sites but when the substrate approaches enzyme, due to conformational changes active site formation is induced.
18. Enzyme kinetics is process to measure rate of enzyme catalyzed reaction.
19. Michaelis and mention derived a mathematical equation for calculation of rate of enzyme catalyzed reaction.
20. Inhibitor molecules reduce rate or block enzyme catalyzed reaction.
21. In competitive inhibition, inhibitor molecule binds to active site and prevents enzyme substrate complex formation.
22. In uncompetitive inhibition, the inhibitor molecules bind to enzyme substrate complex and prevent formation of products.

9.9 GLOSSARY

- **Activation energy:** Activation energy is the minimum amount of energy that must be provided for compounds to result in a chemical reaction.
- **Catalyst:** Catalyst is molecule or substance which increases rate of a reaction without itself being consumed
- **Chiral C atom:** chiral carbon is a carbon atom that is attached to four different types of atoms or groups of atoms
- **Cofactor:** A cofactor is a non-protein compound or metallic ion that is required for an enzyme's role as a catalyst
- **Denaturation:** Denaturation involves the breaking bonds within a protein molecule that are responsible for the highly ordered structure of the protein in its natural state
- **Histones:** Histones are a family of basic proteins that associate with DNA in the nucleus and help condense it into chromatin
- **Peptide bond:** Peptide bond is a chemical bond formed between two molecules when the carboxyl group of one molecule reacts with the amino group of the other molecule, with release of one molecule of water.
- **Prosthetic group:** A prosthetic group is the non-amino acid component that is part of the structure of the heteroproteins or conjugated proteins
- **Start Codon:** AUG is start codon through which the process of protein synthesis begins.
- **Stop codon:** UAA, UAG and UGA are three stop codons, when any of these is read on mRNA the process of protein synthesis is terminated.

9.10 SELF ASSESSMENT QUESTIONS

9.10.1 State whether following statements are true or false

1. Essential amino acids are synthesized inside living organisms.
2. Protein synthesis is also known as translation.
3. Amino acids are amphoteric in nature
4. Amino acids have comparatively low melting point
5. Two subunits of prokaryotic ribosome are represented by 40S and 60S
6. Value of V_{max} increases in competitive enzyme inhibition.
7. Uncompetitive inhibition is rare in one substrate reaction and common in bi-substrate reaction.
8. In case of irreversible inhibition, the inhibitor binds to enzyme through covalent bond.
9. Enzymes are specific in their activity.
10. Enzyme increases rate of reaction but do not influence equilibrium of reaction.
11. Globulin proteins are water insoluble.
12. Amino acids get decomposed when heated to very high temperatures.
13. Complete proteins contain all essential amino acids.
14. Fibrous proteins are spherical in shape.
15. Cofactor is defined as non protein part of protein.

9.10.1 Answers Key: 1- False 2-True, 3- True,4- False, 5- False,6-False, 7-True, 8-True, 9-True, 10-True, 11- False, 12- True, 13- True, 14- False, 15-True.

9.10.2 Fill in the following blanks

1. In competitive inhibition the inhibitor molecular binds to _____.
2. Lock and key mechanism of enzyme action was given by _____.
3. Value of V_{max} is _____ during uncompetitive inhibition.
4. Cyanide binds to _____ on enzyme cytochrome oxidase.
5. The term enzyme was coined by _____ in 1878.
6. Amino acids are stereospecific due to presence of _____ carbon atom.
7. Enzyme _____ catalyze transfer of functional group from one compound to other.
8. Keratin is an example of _____protein.
9. Based upon shape proteins are classified as _____and_____.
10. The process of formation of RNA from DNA is known as_____.

9.10.2 Answer Key: 1- Active site , 2- Emil Fisher , 3- Decreases , 4- Allosteric site , 5- Kuhne , 6- Chiral, 7- transferases, 8- Structural, 9- Globular , fibrous, 10- Transcription.

9.10.3 Multiple choice questions

1. Which of the following is correct about competitive inhibition?

(a) Inhibitor binds to active site (b)V_{max} remains unchanged
(c) Km increases (d) All the above are correct

2. Holoenzyme is correctly represented by
   (a) Enzyme – prosthetic group (b) Enzyme + prosthetic group
   (c) Enzyme + substrate (d) Enzyme + active site

3. Induced fit model of mechanism of enzyme action was gene by
   (a) Emil Fisher (b) George Mendel
   (c) Daniel Koshland (d)Har Govind Khurana

4. Relenza drug is an example of
   (a) Non-competitive inhibitor (b) Competitive inhibitor
   (c) Uncompetitive inhibitor (d) None of the above

5. Methotrexate is an example of
   (a) Non-competitive inhibitor (b) Competitive inhibitor
   (c)Uncompetitive inhibitor (d) None of the above

6. In non competitive inhibition the inhibitor molecule binds to
   (a) Active site (b) Substrate
   (c) Allosteric site (d) Product

7. Turnover number stands for
   (a) Number of substrate molecule converted to product per unit time
   (b) Number of inhibitor molecule converted to enzyme per unit time
   (c) Number of enzyme molecule converted to product per unit time
   (d) Enzyme substrate formed per unit time

8. NAD stands for
   (a) Nicotinamide adenine dinucleotide (b) Nitroamide adenine dinucleotide
   (c)Nicoamide adenosine diphosphate (d) Nitroamide adenine diphosphate

9. Which of the following of an example of simple protein
   (a) Albumin (b) Globin
   (c) Histones (d) All the above

9.10.3 Answers Key: 1-d , 2-b , 3- c , 4- b , 5-b , 6-c , 7- a , 8-a , 9- d

9.10.4 Very short answer type questions

1. Define active site?

2. Give two examples of competitive inhibitor of enzymes?
3. What are cofactors?
4. Name three stop codons?
5. What are derived proteins?
6. What do you understand by polypeptide chain?
7. Differentiate between exoenzymes and endoenzymes?
8. How does reversible enzyme inhibition differ from irreversible enzyme inhibition?
9. Differentiate between complete and incomplete proteins?
10. Mention the function of transporter proteins?
11. What roles do structural proteins play?
12. Give two examples of simple proteins?
13. What is peptide bond?
14. Mention the function of A site in protein synthesis?
15. Identify the difference between ribosomes of prokaryotes and eukaryotes?
16. Name two Sulphur containing amino acids?
17. Draw general structure of amino acids?
18. How does basic amino acid differ from acidic amino acids?

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9.13 TERMINAL QUESTIONS

9.13.1 Short answer type questions

1. Enlist characteristic features of amino acids
2. Differentiate between primary and secondary derived proteins
3. Briefly describe about prokaryotic and eukaryotic ribosomes
4. Mention about role of different initiation and elongation factors involved in protein synthesis?
5. Enlist general properties of enzymes

6. Write short note on enzyme kineties?
7. What type of reaction is catalyzed by enzyme transferases?
8. Differentiate between primary and secondary rived proteins?
9. Mention characteristic features of histone proteins?
10. Classify proteins based upon their function?
11. Differentiate between essential and non essential amino acids?

9.13.2 Long answer type questions

1. Classify enzymes based upon the biochemical reactions catalyzed by enzymes?
2. Describe the process of protein synthesis?
3. With example of lock and key and induced fit model explain mechanism of enzyme action?
4. Provide a comparative account of completive and non-completive enzyme inhibition?
5. Describe in detail structural organization (levels) of proteins?
6. Giving suitable example explain about conjugated proteins?
7. Provide a detailed classification of amino acids?

UNIT-10-SECONDARY METABOLITES AND DEFENSE COMPOUNDS, FLOWERING AND FRUIT REPINING

Contents:

10.1OBJECTIVES

- To study about different types of secondary metabolites produced in plants.
- To identify function of secondary metabolites and their specific role in plant defense.
- Study about the physiology of flowering in plants.
- Study about factors influencing fruit ripening and biochemical changes involved.

10.2 INTRODUCTION

Metabolism refers to sum of catabolic and anabolic reactions occurring inside a living cell/organism. As a result of metabolic reactions going inside a living cell a wide range of products are formed known as metabolites. Largely, metabolites are classified as primary and secondary metabolites. In general, primary metabolites are essential for growth, development and reproduction. Secondary metabolites are basically derived from primary metabolites and have been reported to possess several functions and biological activities. A wide range of secondary metabolites are produced by plant species belonging to chemical groups such as alkaloids, terpenoids, flavonoids, non-protein amino acids, phenolics, quinones, amines etc.

Functions of secondary metabolites:

1. Secondary metabolites possess antibacterial, antifungal and antiviral activity.
2. Secondary metabolites are known to possess UV radiations absorbing components which protect plant leaves from damage
3. Secondary metabolites act as defensive compound against herbivores
4. Secondary metabolites specifically responsible for colour of flower and fragrance help in attracting insects which further mediate pollination.
5. Secondary metabolites help in plant response to abiotic stress.
6. Secondary metabolites are also known to attract pollinators

10.3 SECONDARY METABOLITES AND DEFENSE COMPOUNDS

10.3.1Cutin

Cuticle is a protective covering found on surface of plant organs. Cuticle comprises of three-layer, outer layer is of wax, middle layer is known as cuticle proper and is made up of cutin and third layer is cuticular layer which is a mixture of cutin and polysaccharides. Cutin is a fatty (lipid in nature) substance deposited upon surface of epidermal cell wall. Cutin is comprised of hydroxy and epoxy fatty acids and are present on ariel parts of plants including stem, leaves, flower, fruits and seeds. Cutin is a polymer of long chain fatty acids (16:0 and 18:1) attached to each other by ester linkages. Cellulose or pectic substance of primary or secondary wall is transformed in to cutin by a process known as cutinization. The cutin layer is impermeable to water. Cutin is resistant to microorganisms hence provide protection from pathogens.

10.3.2 Suberin

Suberin is a polymer which is made up of long chain hydroxy and dicarboxylic acids, alcohols, phenolic compounds and wax. Suberin is water impermeable. Suberin is present in cell wall of peri-dermis, endodermis as well as in seed coats. The abundance of suberin varies from species to species. Presence of suberin in casparian strips limits transport of water and solutes hence protects plant vascular tissues from microbial pathogens. Suberin deposition also helps in wound healing of plant tissues. Suberin develops as a sealing tissue when there is a wound on plant surface or at the site from where leaf abscission has occurred. Suberin is found to be present in outer cell walls of underground plant organs. Overall function of suberin can be summarized as protection against pathogen, wound healing, prevention of loss of water and protection against heat exposure. There are two main domains recognized considering the structure of suberin. Polyaromatic domain in primary cell wall and poly aliphatic in between primary cell wall and cell membrane. The aliphatic component is insoluble polyester comprising of fatty acids, glycerol, hydroxy fatty acids, oxygenated fatty acids, unsubstituted fatty acids.

10.3.3 Waxes

The surface of leaves, flower and fruits is covered by wax. Plant derived wax are dominated by unesterified hydrocarbon as compared to esters. Major constituents of lipid plant wax are alkanes, alkyl esters, fatty acids, fatty alcohols, fatty aldehyde, ketones, diketones, tri terpinols. The composition of wax varies from species to species and also by the habitat (geographical region/environment) in which the plant is cultivated.The intensity as well as duration of light (sunlight) also affects accumulation of wax in plants.Waxes prevents dehydration and evaporation. The presence of wax over the surface of leaves also provides protection from insects and diseases. Presence of wax permits controlled release of volatile compounds to prevent pests or attract pollinators. Wax can also serve as energy storage material especially in microscopic aquatic plants. Commercial use of plant derived wax includes manufacture of polish, crayons, candles, ink, cosmetics. Plant wax are also utilized to synthesize edible coatings for food items. Presence of wax protects plant leaves from damage due to frost (cold tolerance). Along with this presence of wax also provides protection from ultraviolet radiation.

Some common plant wax

(1) Carnauba: Leaves of carnauba palm (*Copernicia cerifera*) grown in Brazil possesses thick wax coating. It is also known as palm wax. The wax is harvested from dried leaves and utilized in wide range of products.

(2) Soy wax: Soy wax is obtained from soyabean oil. It is commercially utilized in candle production.

(3) Candelilla wax: It is obtained from leaves of *Candelilla* which is a native shrub to northern Mexico and South western US.

(4) Jojoba wax: Jojoba wax is obtained from jojoba plant (*Simmondsia chinensis).* The wax is resistant to oxidation and is utilized in production of cosmetic products.

10.3.4 Terpenes

Terpenes are regarded as the largest and most diverse group of secondary metabolites found in plants. Terpenes are plant secondary metabolites derived from 5-C isoprene units.Different terpenes have different arrangement of isoprene units (Table 1).

Table 1: Classification of terpenes based upon isoprene unit

	Class	No. of isoprene unit	Characteristic feature	Example
1.	Hemiterpenes	1	Isoprene is itself hemiterpene along with prenol and iso valeric acid. They act as signaling defense molecule.	Isoprene
2.	Monoterpenes	2 ($C_{10}H_{16}$)	Common in plants families from which essential oil are extracted (Lamiaceae, Dutaceae, etc.)	Geraniol Terpineol Limonene
3.	Sesquiterpenes	3 ($C_{15}H_{24}$)	More than 200 structural types of sesquiterpenes are known. Possess several biological activities.	Humulene Farnesol
4.	Diterpenes	4 ($C_{20}H_{32}$)	Active constituent of medicinal plants derived from geranyl pyro Phosphate.	Cafestol Taxadiene (Precursor of taxol)
5.	Sesterterpenes	5($C_{25}H_{40}$)	Are rare compounds. Are also reported to be found in marine algae. Possess anticancer activity.	Leucosesterterpenone and leucosterlactoneisolated from*Leusceptrumcanum*
6.	Triterpene	6 ($C_{30}H_{48}$)	Represent lipid content of plants	Squalene
7.	Sesquiterpenes	7 ($C_{35}H_{56}$)	Microbial is origin	Ferrugicadiol
8.	Tetraterpenes	8 ($C_{40}H_{68}$)	Bicyclic α, Beta carotene acyclic lycopene	Lycopene, β-carotene

Characteristic features of terpenoids: -

1. They are colour less liquids or solids.
2. Possess smell or flavors.
3. Terpenoids are insoluble in water.
4. Terpenoids are soluble in alcohols and other organic solvents.
5. Terpenoids are generally optically active.
6. Terpenoids are oxidized by oxidizing agent.

Terpenoids are classified into different types based upon the number of rings present.

a. Acyclic terpenoids are open chain compounds. Eg: - Geraniol, **Linalol**.
b. Monocyclic terpenoids possess one ring. Eg: - Menthol, Menthone, Terpinolene.
c. Terpenoids containing two rings are called as Bicyclic terpenoids. Eg: - Thujone, Camphor.
d. Terpenoids containing three rings are called tricyclic terpenoids.

Functions of terpenes:

1. Monoterpenes possess various medicinal properties. Camphor menthol are used as anti-itching agent and counterirritants analgesics. Monoterpenes are also used as anthelmintics.
2. Sesquiterpenes exhibit anti-fungal, anti-bacterial and anti-protozoan activities.
   Other biological activities of sesquiterpenes include diuretic, analgesic and anti-inflammatory activity.
3. Diterpenes are also reported to possess several biological and pharmacological activities namely anti-fungal, anti-inflammatory, anti-bacterial, analgesic, anti-protozoal activity.
4. Gibberellins, a well-known plant hormone which helps in seed germination and growth of seedling are also diterpenoid acid.
5. Triterpenes, β-Boswellia acid and α-Boswellia acid are reported to possess anti-inflammatory activity. Quassia (product triterpene) acts as insecticide.

10.3.5 Glycosides

Glycoside is defined as a organic compound in which a sugar is attached to other functional group through a glycoside bond. The non-sugar part is known as aglycon or genie. Glycosides are synthesized as well as hydrolyzed by the action of enzymes. Glycosides are colourless, soluble in water and also in alcohol but are insoluble in organic solvent such as benzene and ether. In general glycosides possess bitter taste. Glycosides can be crystalline or amorphous. Glycosides are also classified depending upon the glycone (sugar) part for example when glycone part is glycose then molecule is known as glycoside. When the sugar molecule is fructose the compound is known as fructoside.Rhamnosides contain rhamnose sugar.Based upon the linkage between the glycone (sugar) part and aglycone (non-sugar) part glycosides are classified into different types (Table 2).

Table 2: Classification of glycosides

	Type of glycoside	Linkage	Example
1.	O-glycoside	Sugar part is linked with oxygen atom of aglycone.	Cardiac glycoside, sennosides
2.	S-glycoside	Sugar part is attached to S atom of aglycone part of glycoside.	Sinigrin

3.	N-glycosides	Glycosides in which sugar part is linked to N atom of amino group of aglycone part.	Nucleosides
4.	C-glycosides	Sugar part is directly linked to carbon atom of aglycone part.	Anthraquinone glycosides

Glycosides are also classified as primary or secondary glycosides. Glycosides which are originally present in plants are known as primary glycosides. Eg: - Purpurea A. and secondary glycosides are derived from primary glycosides by removal of one sugar molecule. Eg: - Digitoxin. Several functions of glycosides are known. Glycosides possess medicinal properties, glycosides also serve as source of energy. Glycosides convert toxic molecules to less toxic. Glycosides are also involved in regulatory and protective function.

10.3.6 Phenolic compounds

Among wide spread groups/classes of secondary metabolites produced in plants phenolics are among the largest class of secondary metabolites (in plants). Phenolic secondary metabolites produced in plants range from simple to complex with a characteristic feature of presence of phenolic group. Different classes of phenolic compounds include simple hydroxy benzoic acid, free and conjugated hydroxy cinnamic acid, coumarins, flavonoids. The largest group of phenolic compounds is flavonoids with more than 2000 compounds to be known.Most common flavonoids are anthocyanins, flavones and flavanols. There are several characteristic features of phenolic secondary metabolites such as they contain one or more phenolic groups, they are known to contribute to color, flower of plant products and they possess several biological activities. There are two common pathways of synthesis of phenolic compounds, Shikimic acid pathway and Malonic acid pathway. Phenylalanine is a common intermediate in biosynthesis of phenolic secondary metabolites. Synthesis of phenolic secondary metabolites occurs at different sites in plant cells including chloroplast and membrane of endoplasmic reticulum.Phenolic compounds act as defense compounds. They provide protection against pathogens and herbivores. Inherent anti-microbial activity and anti-nutritional or unpalatable properties are main reason for phenolic secondary metabolites to act as defensive compounds in plants.

Phytoanticipins and phytoalexins

Phytoanticipins are low molecular weight compounds having antimicrobial activity. These compounds are either present in plants before infection or are synthesized after infection from preexisting compounds.

Some examples of preformed phenolic compounds include:

1. Catechol, protocatechuic acids synthesize in onion and acts against *Colletotrichum circienans*.
2. Proanthocynidins in barley provides protection against *Fusarium spp.*
3. Benzaldehyde in potato provides protection against *Botrytis cineria*

Phytoalexins is another class of low molecular weight compound which also have antimicrobial activity. These compounds are synthesized and accumulated in plant cells after the invasion of pathogens/microorganisms.

Some example of phenolic compounds synthesized after attack of pathogen.

1. Compound pisatin is synthesized in Pea plant in response to pathogen ***Nectriahaematococes***.
2. Iso flavonoid is a defense compound produced in green bean against *Colletotrichum* ***lindemuthianum***.
3. Luteolini din in sorghum is a defensive compound against *Colletotrichum* ***gramine*** *cola.*

Function of phenolic compounds in plants

i. They provide colour, flavor or taste.
ii. Many phenolic secondary metabolites possess anti-inflammatory activity.
iii. Compounds genistein and daidzein are known as for their phytoestrogenic activity.
iv. Phenolic compounds also act as antioxidants(flavonoids), insecticides (common example naringenin).
v. Gallic acid is a well-known phenolic compound found to be present in plant kingdom. Several biological activities of gallic acid include anti-inflammatory activity, anti-fungal, anti-viral and anti-tumor activity.
vi. Simple phenolic compounds present in *Capsicum spp., Cynara Scolymus, Ulmaria* are reported to possess anti-inflammatory, analgesic activity, diuretic activity.

10.3.7 Alkaloids

Alkaloids arenaturally occurring organic compounds containing at least one nitrogen atom in their structure (heterocyclic ring).Leguminosae, Papaveraceae, Pubiaceae, Solanaceae, Ranuculaceae are among common plant families known for production of alkaloids along with members of family Berberidaceae are high alkaloid producing plants. Table 3 depicts some major alkaloids, their source and major properties.

Table 3: Some example of plant alkaloids

Alkaloid	Plant species	Properties
Nicotine	Nicotiana tabacum and other Nicotiana species	Tranquilizing properties, Additive toxic
Caffeine	Coffer spp., Camellia, sinensis, cola acuminata	Diuretic, Stimulatory effect on respiratory, CNS.
Vinblastine	Catharanthus, Roseus G.	Anti-Diabetic, Disinfectant

Classification of alkaloids:

1. **True alkaloid**:
   Also known as heterocyclic alkaloids due to presence of heterocyclic ring. Heterocyclic nitrogen is derived from amino acids. Eg: Pyrrolizidine derivatives
2. **Pseudo alkaloids**:
   Pseudo alkaloids are not derived from amino acids and mainly include steroidal and terpenoid alkaloid. Eg:Caffeine
3. **Proto alkaloids**:
   Proto alkaloids are simple amines is which N atom is not in a heterocyclic ring. They are also called biological amines or amino alkaloids. Eg: Ephedrine, Colchicine

Function of alkaloids: -

1. Protect plants against insects and herbivores due to their toxic/poisonous nature.
2. Several alkaloids are known for their medicinal properties.
   Quinone is known as antimalarial agent.
   Vincristine possesses anticancer activity.
   Mydriatics is a known atropine.
   Morphine, codeine are known as analgesic.

   Alkaloids are also known for their other biological activity such as anti-inflammatory activity, anti-pyretic etc.

3. Alkaloids can serve reserve substance and supply N and other elements to plants.

10.4 FLOWERING AND FRUIT REPINING

10.4.1Photoperiodism

Garner and Allard in 1920 introduced the term photoperiodism.They observed Glycine max(Soyabean) and Maryland Mammoth to follow seasonal pattern of following. Plants of soyabean exhibited flowering in late summer (irrespective of the time when seeds were sown).

Based upon photoperiod plants are characterized into three groups (Fig. 10.1):

1. Short Day flowering plants (SDP)
2. Long day plants
3. Day neutral plants

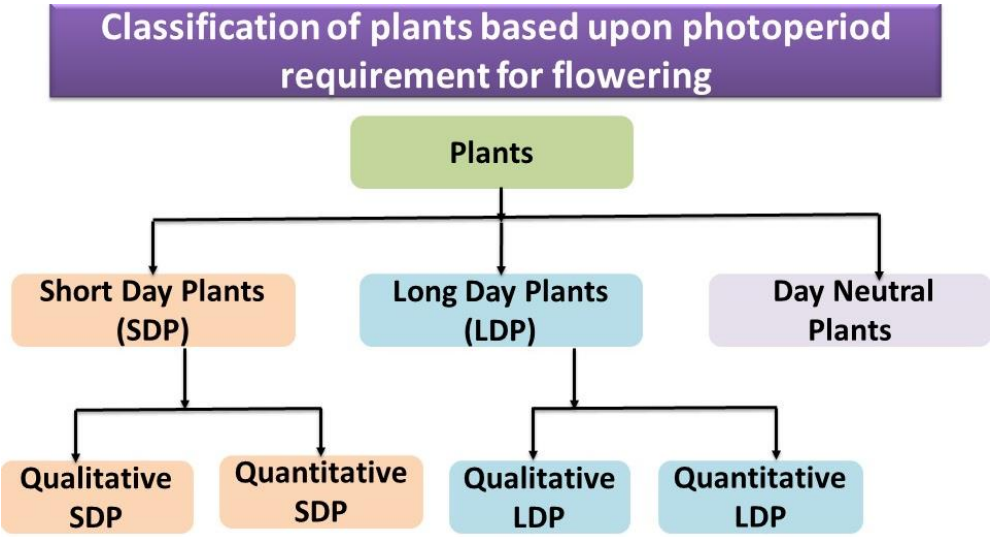


Figure.10.1: Classification of plants based upon photoperiod requirement for flowering

Short day flowering plants

Short day flowering plants exhibit flower when length of day is less than a critical period. If length of day (exposure to light) exceeds a critical period then flowering is inhibited. These plants are also known as long night plants. Fig 10.2 depicts how photoperiod influences flowering in SDP.

Characteristics feature of SDP:

1. A continuous, uninterrupted long period of darkness is required for flowering to occur in SDP.
2. Flowering is inhibited in SDP if continuous period of darkness is inhibited by light.
3. Short day flowering plants can be made to flower under long to day conditions, if provided with sufficient period of darkness.
4. Short day plants also flower under complete period of darkness with sucrose provided externally.

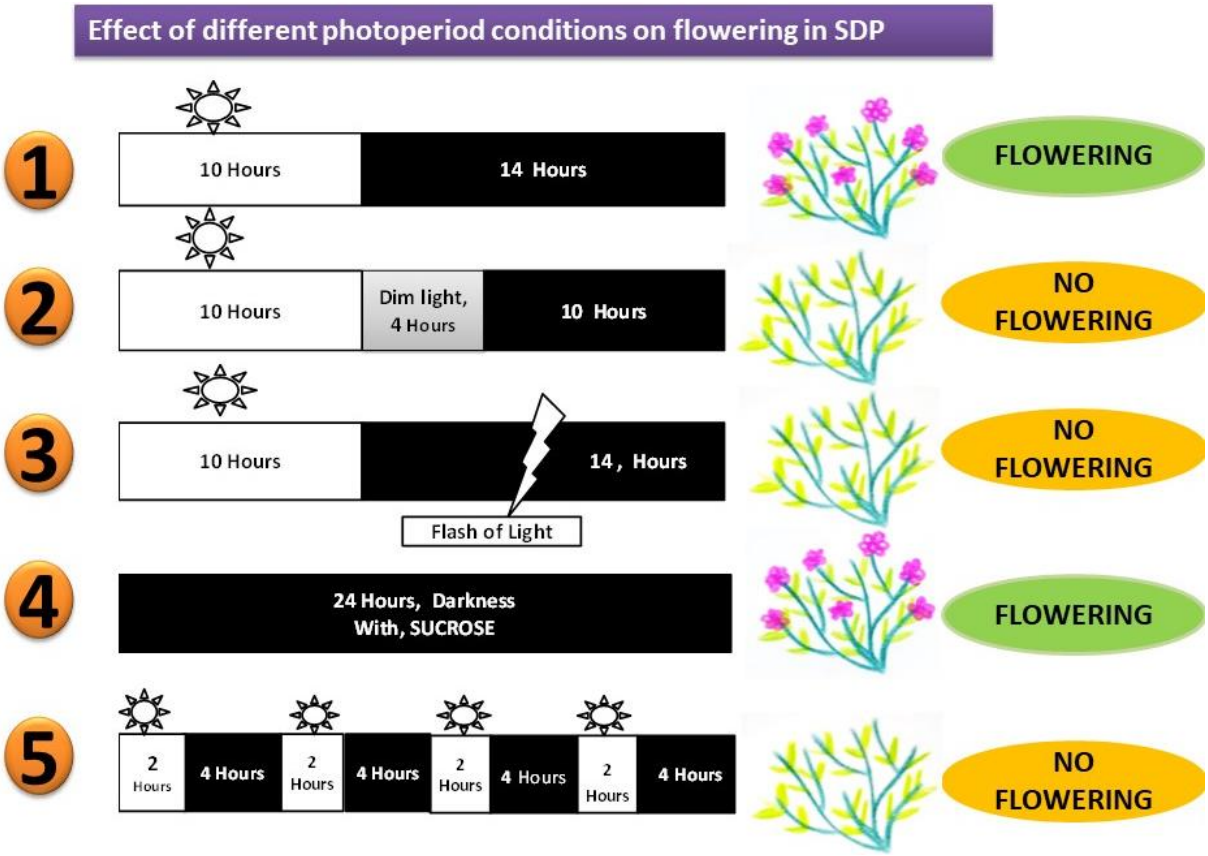


Fig 10.2: Flowering response of short day plants under different photoperiod conditions

1. *SDP flower under longer period of darkness.*
2. *Flowering is inhibited in absence of sufficient period of darkness.*
3. *Flowering is inhibited if period of darkness is interrupted by flash of light.*
4. *SDP exhibited normal flowering under complete period of darkness with sucrose provided externally.*
5. *No flowering occurs if the period of darkness provided is not of continuous.*

Table 4: Different types of short day plants

Category	Characteristic feature	Example
Qualitative short day plants	This category SDP show flowering only under short day conditions . They are also known as obligatory short day plants.	*Fragoria*(strawberry), *Zeamays* , *Coffea arabica*
Quantitative short day plants	Optimum flowering in these type of SDP occurs under short day conditions. However, these plants can also be made to flower under long day conditions.	*Gossypium hirutum* (cotton)
Short long day plants	These plants flower when short	*Trifolium repens* (white clover)

	day conditions are followed by long days.	

Long Day flowering plants

Long day flowering plants flower under longer period of light (photoperiod). Day length longer than a critical period is required for flowering in LDP.In these plants shorted period of darkness is more crucial than longer period of light because of LDP are exposed to longer duration of darkness flowering is inhibited. These plants are also known as short night plants. Fig 10.3 depicts how photoperiod influences flowering in LDP.

Characteristics features of LDP:

1. Long day flowering plants show best flowering under continuous light.
2. Flowering is inhibited by long period of darkness.
3. These plants can also flower under short days if period of darkness is interrupted by light.
4. Long day flowering plants will flower if alternate period of light and darkness is provided.

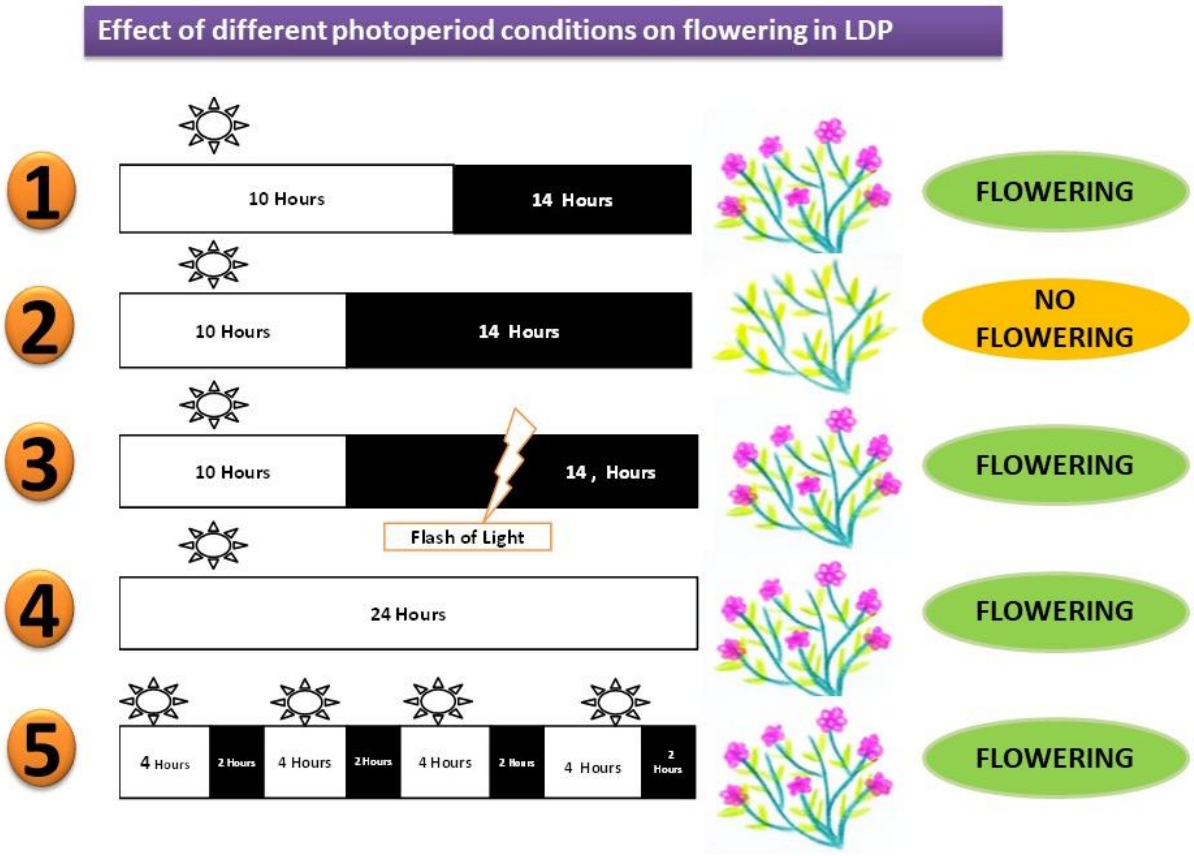
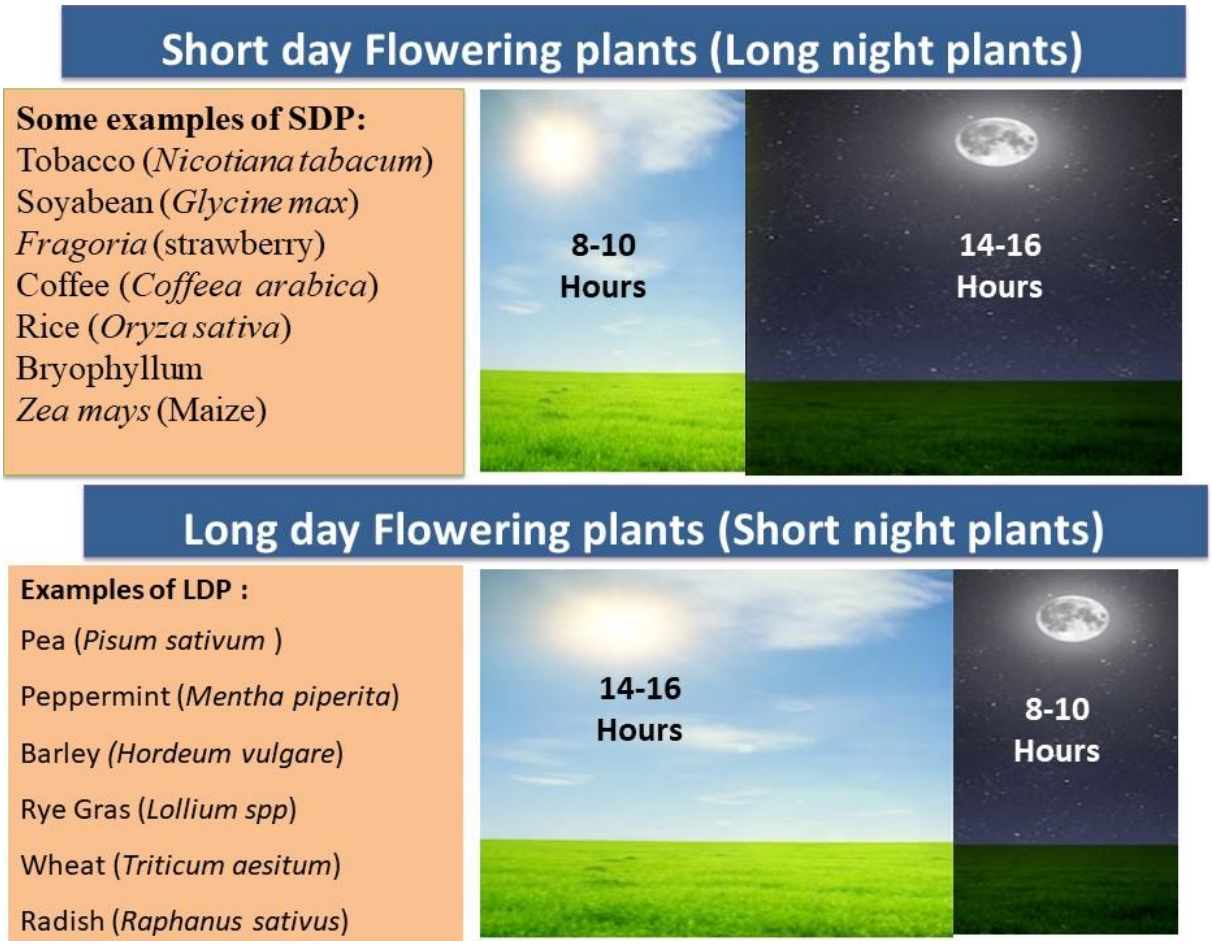


Fig 10.3: Flowering response of long day plants under different photoperiod conditions

1. *Flower occurs when exposed to longer photoperiod.*
2. *Flowering is inhibited by longer period of darkness.*
3. *Flowering occurs if longer period of darkness is interrupted by flash of light.*
4. *Flowering occurs under continuous period of light (no darkness).*
5. *Flowering occurs when alternate period of light and darkness is given to plants.*

Table 5: Different types of long day plants

Category	Characteristic feature	Example
Qualitative long day plants	This category SDP show flowering only under short day conditions . They are also known as obligatory short day plants.	*Fragoria*(strawberry), *Zeamays* , *Coffea arabica*
Quantitative long day plants	Optimum flowering in these type of SDP occurs under short day conditions. However, these plants can also be made to flower under long day conditions.	*Gossypium hirutum* (cotton)
Short long day plants	These plants flower when short day conditions are followed by long days.	*Trifolium repens* (white clover)



Photoperiodic induction

The stimulus for flowering in perceived by leaves. This phenomenon was demonstrated by M.K. Chailakhyan. In the experiment study (Fig. 10.4) the plant chrysanthemum was utilized leaves from the upper parts of the plants were removed (defoliated) and such as plants with no leaves in upper regions were divided into four groups. Each group was exposed to specific photoperiod and following observations were obtained.

I. **Group A:** The complete plants were exposed to long day (longer photoperiod) and no flower occurred.
II. **Group B:** The upper portion of plants with no leaves was exposed to longer photoperiod and lower portion of plant was given short day treatment. Flowering occurred in plants.
III. **Group C:** The upper portion of plants (with no leaves) was given short day treatment. No flowering was obtained in the plants.
IV. **Group D:** the entire plants were exposed to short day treatment. Flowering occurred after this photoperiod treatment.

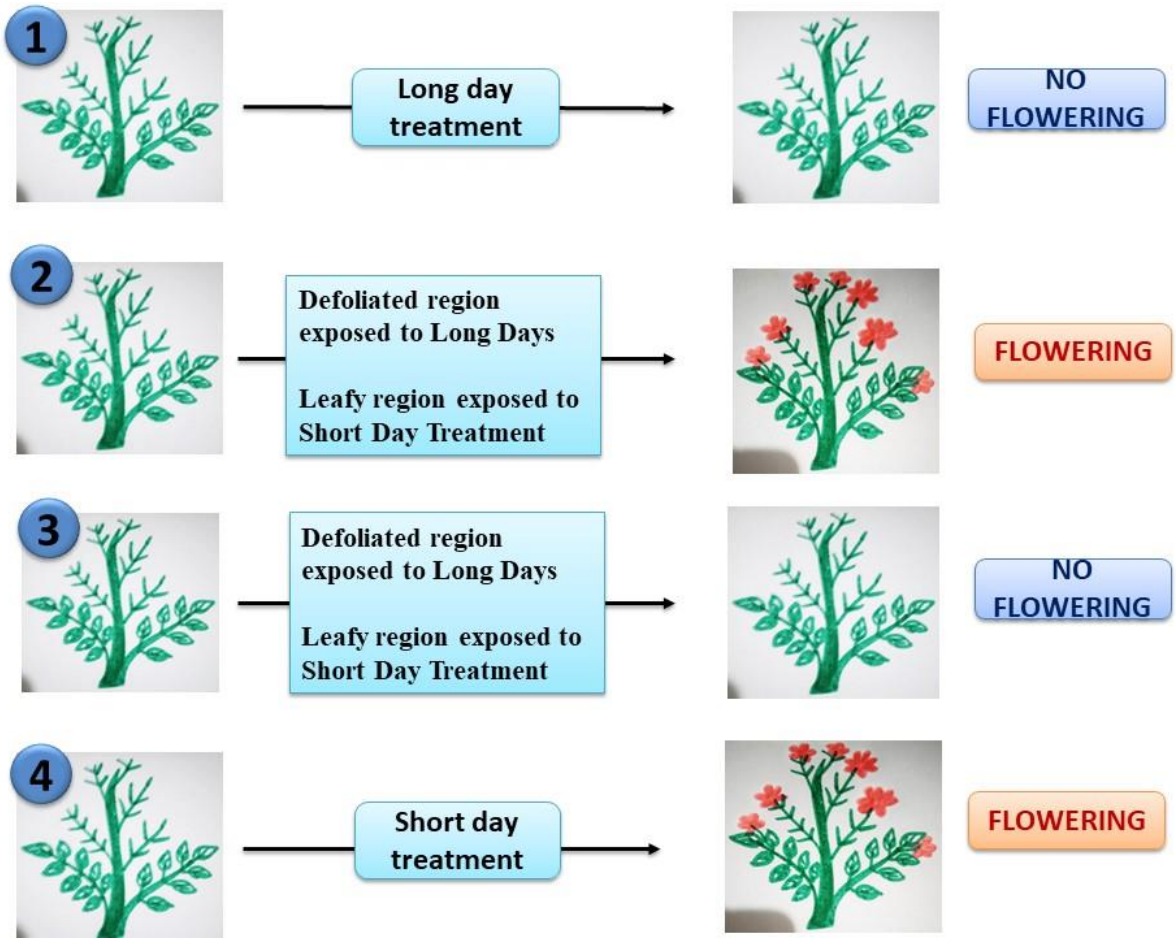


Fig 10.4: Experimental study to illustrate leaves to perceive / respond to flowering stimulus

The above observations proved that leaves are the organ of plant which perceive stimulus for flowering. *Chrysanthemum* is a short-day flowering plant and as we can see in above four experimental setup flowering occurred only when the part of having leaves was exposed to short day treatment. This confirmed that stimulus for flowering in perceived by leaves which in transferred to upper parts of plant for flowering to occur.

Another experiment (Fig. 10.5) was conducted to confirm that even a single leaf in sufficient to perceive the stimulus for flowering. The plants were divided into three categories A, B, C. In category A no change/defoliation was done. Plant of category B were completely defoliated that means all the leaves were removed and plants of category C all leaves were removed except one.Plants of all three categories (A, B and C) were exposed to short day treatment which is required by chrysanthemum plants to flower.As expected, flowering occurred in category A plants and no flowering was obtained in category B plants (because of absence of leaves). Also flowering occurred in category C plants which contained only one leaf. This experimental study also confirmed that flowering that flowering stimulus is perceived by leaves and in transferred to other parts for flowering to occur.

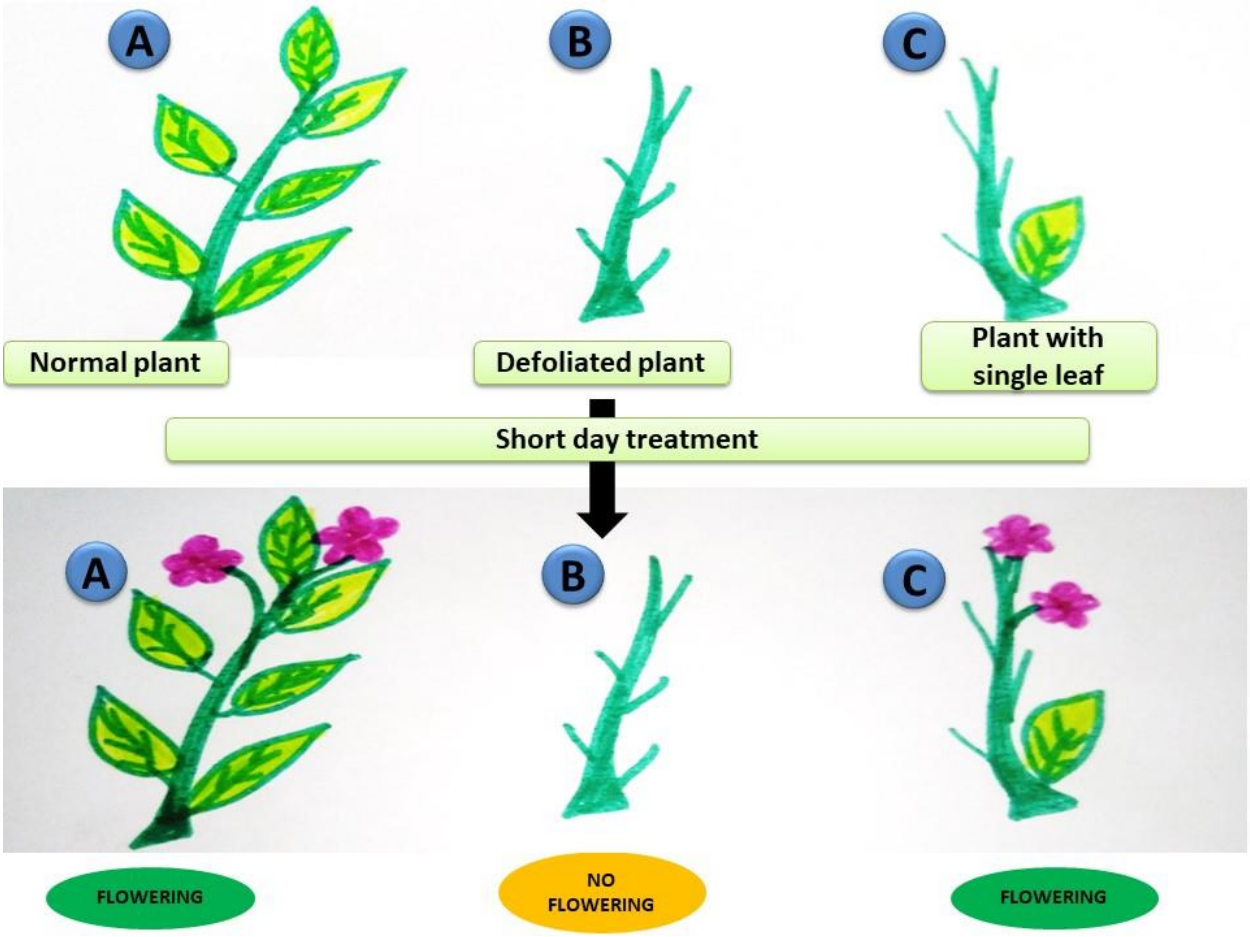


Fig 10.5: Experimental study illustration leaves perceive flowering stimulus

Phytochrome

Phytochrome is a pigment found to be present in plants which is known to control development of plants. These are two known forms of phytochrome (Fig. 10.6): P_R and P_{FR}. P_R is type of phytochrome which absorbs red light and P_{FR} is type of phytochrome which absorbs far red light. Both these forms are interconvertible. P_R is biologically inactive and P_{FR} is biologically active, phytochrome regulation various processes in plants such as unfolding of leaves, pigmentation, hypocotyl hook opening and photoperiodism.

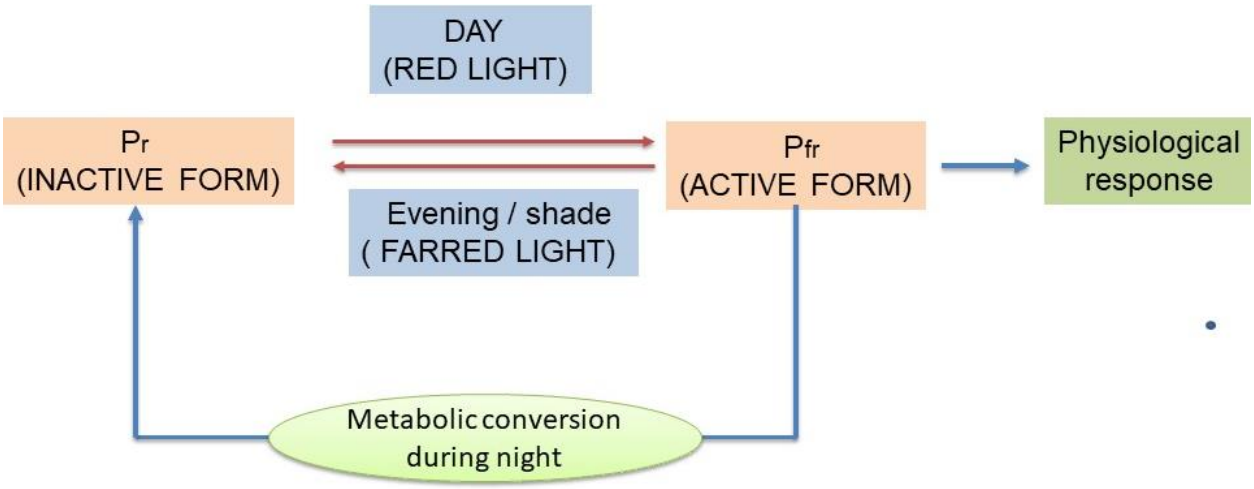


Figure. 10.6: Summary of different types phytochrome in plants

Effect of phytochrome on flowering in SDP:

As already mentioned, short day plants require a crucial longer period of darkness for flowering. If this period of darkness is interrupted by red light flowering is inhibited in SDP. It means that red light has inhibiting effect on flowering in SDP. If treatment with red light is followed by far red light, than the effect of red light is compensated / neutralised and flowering occurs.It a short-day plant in given alternative treatment of red light and far-red light that the effect of treatment given in last will be expressed (Fig.10. 7).

Effect of phytochrome on flowering in LDP:

As we have already studies that flowering in long day flowering plants is inhibited by longer period of darkness. If long day plants are exposed to longer period of darkness they will fail to flower. However, if this longer period of darkness is interrupted by red light. The plants will exhibit normal flowering because the continuous period of darkness is not maintained and the period of darkness fails to exert its inhibiting effect on flowering. But if exposure to red light is followed by exposure to far red light than red light loses its effect and period of darkness maintains its inhibitory effect resulting in no flowering. As in the case of short-day plants if alternative treatment of red and far-red light is given than effect of last treatment will be seen on flowering in long day plants.

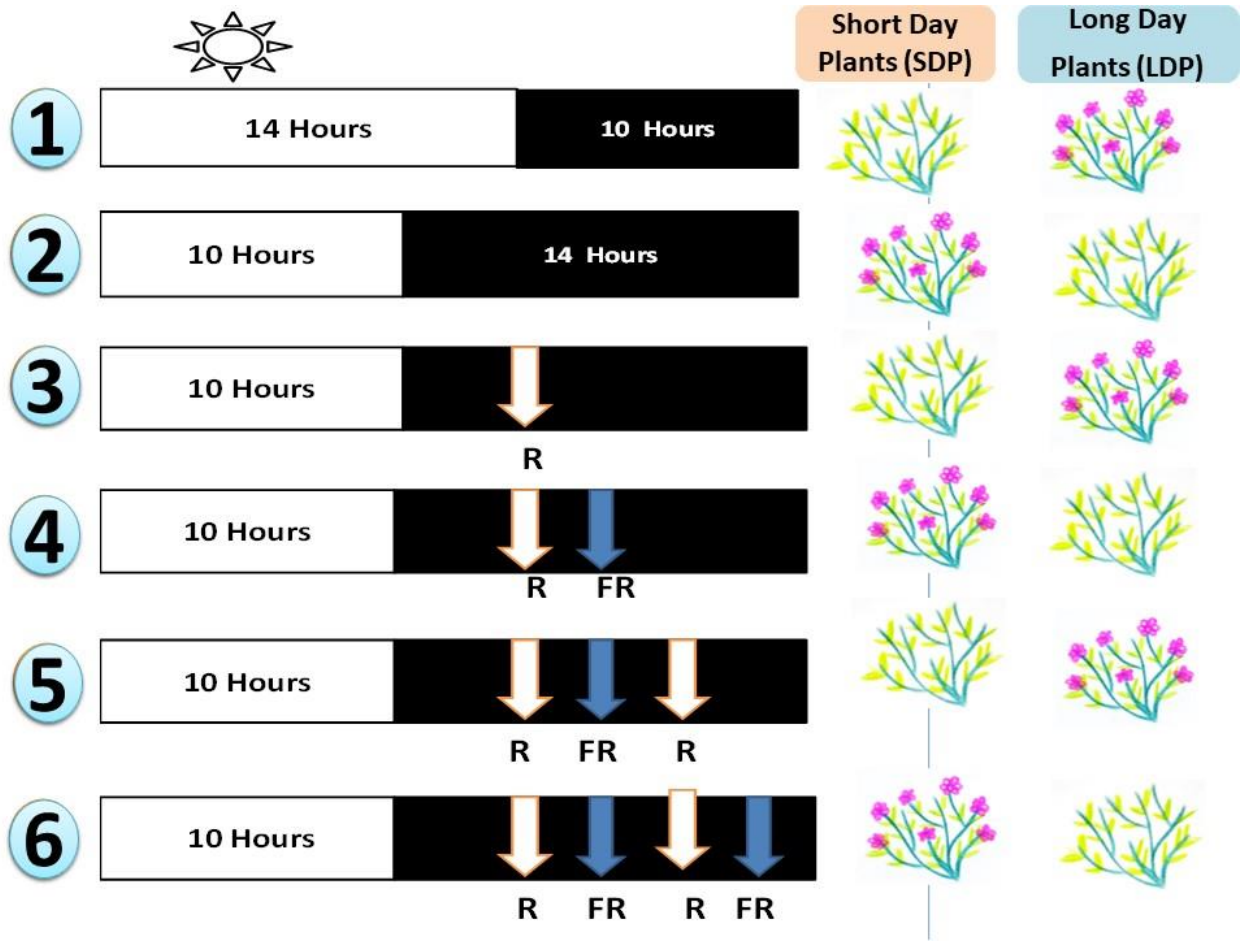


Fig. 10.7: Influence of phytochrome on flowering in plants

10.4.2 Fruit ripeninig

Fruits are defined as ripened ovaries or carpels that contain seeds. Fruit ripening is a process by which raw fruits ripe and become edible. The complete process of fruit formation involves fruit set, fruit development and ripening of fruits. Ripening is the stage which involves various biochemical reactions as a result of which fruit becomes edible and also attractive. Changes in colour, texture, synthesis of aromatic compounds, and accumulation of sugar are among crucial changes occurring during fruit ripening. Fig 10.8 demonstrates summary of biochemical and morphological changes occurring during ripening of fruits.

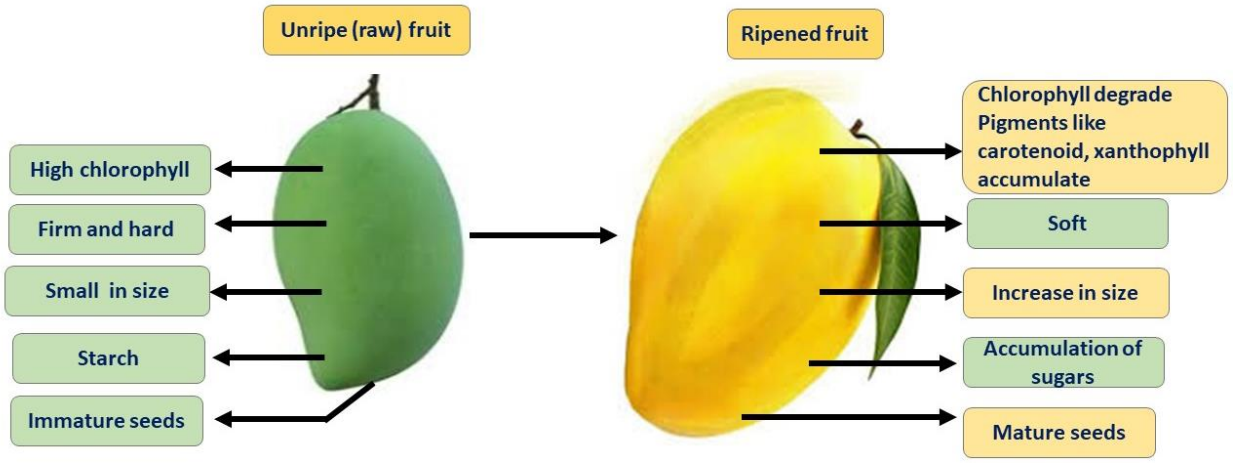


Figure. 10.8: Summary of changes occurring in fruits during ripening

1. Change in flavor:

When fruits ripe their flavor change and they become edible. The main reason of change in flower is conversion of starch into sugar. There are so many fruits which we eat in our daily life and based upon taste we can easily identity difference in flavor of raw and ripe fruits. For example, raw mango, raw guava, raw banana have sour/bitter taste but become sweet when they ripe. (However, there are many fruits which are utilized for several purposes even when they are raw.The most common utilization of raw fruits includes pickle preparation. Mango, lemon are common fruits utilized to prepare pickles in their raw state.) Conversion of starch to sugar is characteristic event for ripening of fruits. Enzymes namely α-amylase, β-amylase, phosphorylase and α-1,6 glucosidase are involved in conversion of starch into sugars.

2. Change in colour (pigmentation)

Another characteristic feature of fruit ripening is change in colour of fruit. Again, there are so many examples where there is a drastic change in colour of unripe (raw) and ripe fruits. Apples are green when raw but becomes red after ripening similarly green coloured raw mango and bananas when ripe the colour changes to orange/ yellow.The change in colour is attributed to accumulation of different pigmentssuch as carotenoid, anthocyanins (Table 6)and degradation of chlorophyll resulting in colour change. Enzyme chlorophyllase catalyzes degradation of chlorophyll. Enzymes phenylalanine ammonia lyase and flavors synthase are the enzymes involved in synthesis of anthocyanins.

Table 6: Different pigments found in ripened fruits

Colour of ripened fruit	**Pigment**
Yellow colour	Xanthophyll flavonoid
Orange	Carotene
Red colour	Lycopene

Purple	Anthocyanin

3. Softening of fruits:

Raw fruits are Ferm and hard but when the fruits ripe they lose their firmness and becomes soft. The firmness of raw fruits is mainly due to pectin, cellulose present in cell wall. Enzymes polygalacturonase brings out hydrolysis of pectin bonds and enzyme methyl esterase pectinase degrades pectin present in cell wall.

Along with depolymerization of pectin, hydrolysis of other polysaccharides of cell wall results in softening of fruits.

4. Seed maturation:

In ripen fruits seeds mature and exhibit ability to germinate.

Another feature of fruit ripening is development of wax on peel. This helps in prevention of dehydration.

Ripening of fruits is accompanied by decrease in organic acid content in fruits. The decrease in amount of organic acids in fruit with ripening is due to increased membrane permeability.

Hormonal control of fruit ripening

Ethylene is hormone known for its function as fruit ripening hormone. Synthesis of ethylene occurs from S- adenosylmethionine through amino cyclopropane -1- carboxylic acid (ACC). The biochemical reactions involved in synthesis of ethylene are catalyzed by enzymes ACC synthase and ACC oxidase. Beside its significant role in flowering ethylene hormone is known to influence other physiological and development plant processes such as leaf abscission, root nodulation, response to environmental stress etc. Beside ethylene, other hormones such as auxin is also involved in processes of fruit ripening.

S- adenosylmethionine (SAM)

↓ ACC Synthase

Amino cyclopropane-1-carboxylic acid (ACC)

↓ ACC oxidase (ACO)

Ethylene

Adverse effects of Ethylene:

Although ethylene is a hormone which is known to support ripening of fruits. However, there are some adverse effects of ethylene also.

1. Since ethylene causes ripening of fruit so it also causes undesired ripening of fruits / vegetables when they are stored. As a result, the storage time (shelf life) of fruits decreases.
2. Effect of ethylene causes abscission of leaves in cauliflower, cabbage and other foliage plants.
3. Loss of green colour in leafy vegetables.
4. Synthesis of phenolic compounds often render undesirable taste to fruit / vegetable.

Climacteric and Non climacteric fruits

Climacteric fruits are those fruits in which rate of respiration rises during process of ripening. Climacteric fruits require ethylene for ripening and ethylene production drastically increases during ripening. These fruits show/ripening even after their harvesting. In maturing fruits, the rate of respiration is low which increases during ripening. Continuous increase in rate of respiration after reaching a climacteric peak again decreases.

Non climacteric fruits are those in which rate of respiration remains steady during ripening process. Non climacteric fruits in general do not require ethylene for ripening with little or no increase in ethylene level during fruit ripening. Also, another difference between climacteric and non-climacteric fruits (which ripe even after they are harvested), non-climacteric fruits dose not ripe once harvested.

Examples of climacteric fruits	Examples of Non climacteric fruits
Papaya, Banana, Tomatoes.	Pineapple, Strawberry, Grapes, Lemon.

10.5 SUMMARY

1. As a result of metabolic reaction occurring inside living cells a large number of metabolites are produced.
2. Primary metabolites produced are essential for survival.
3. Secondary metabolites are derived from primary metabolites.
4. Secondary metabolites responsible for several biological and medicinal properties of plants.
5. Secondary metabolites also serve as defense compounds.
6. Cutin is water permeable and provides protection from pathogen and prevents water loss.
7. Suberin polymer provides protection from heat pathogen and prevents water loss.
8. Plants produce waxes which provide production from insects and diseases.
9. Terpenes are among largest secondary metabolites produced in plant kingdom. Terpenes are derived from 5-C isoprene units.
10. Terpenes possess smell and flower they are water insoluble and solute in alcohol and other organic solvent.
11. Terpenes are classified based upon number of rings present.

12. Glycosides are another major class of compounds. Glycosides are colourless, water soluble and generally possess better taste.
13. Glycosides are classified based upon sugar part present.
14. Another important class of phytocompounds is phenolic compounds which are known to possess several functions and biological activities.
15. Phytoanticipins and phytoalexins are low molecular weight compounds having antimicrobial activity.
16. Alkaloids are organic compounds containing at least one N atom and are classified as true, pseudo and proto alkaloids.
17. Depending upon the photoperiod requirement for flower plants are classified as short-day flowering plant, long day flowering plant and day neutral plants.
18. The stimulus of flowering is perceived by leaves.
19. Phytochrome is a pigment present in plants which controls plant development.
20. Pr is inactive form and Pfr is active form of phytochrome.
21. Red light exerts inhibitory effect on flowering in SDP.
22. Fruits are ripened ovaries or carpels.
23. Various physiological and biochemical changes occur during fruit ripening.
24. Change in colour, flavor and taste, softening of fruits, increase in size and maturity of seeds are major changes occurring during ripening of fruits.
25. Ethylene hormone is known as fruiting hormone.
26. Fruits are classified as climacteric andnon climacteric fruits.
27. Climacteric fruits require ethylene for ripening whereas non climacteric fruits donot require ethylene for ripening of fruits.

10.6 GLOSSARY

- **Alkaloids :** Alkaloids are a class of basic, naturally occurring organic compounds that contain at least one nitrogen atom.
- **Analgesics :** Analgesics are a class of medications designed specifically to relieve pain
- **Antioxidants :** Antioxidants are molecules that fight free radicals in your body.
- **Flavonoids :** are a class of polyphenolic secondary metabolites found in plants,
- **Non-climacteric fruits :** Non-climacteric fruits are characterized by ripening transitions that do not strictly depend on a significant increase in ethylene production and an associated rise in respiration rate
- **Pathogen:** A pathogen is defined as an organism which causes disease to its host. Common pathogens include bacteria, fungi and viruses.
- **Photoperiodism:** Photoperiodism is the functional or behavioural response of an organism to changes of duration in daily, seasonal, or yearly cycles of light and darkness.
- **Phytoalexins:** Phytoalexins are low molecular weight compounds having antimicrobial activity which are produced by plants in response to biotic and abiotic stresses.

- **Phytoanticipins :** Phytoanticipins are antimicrobial compounds found in plants which are synthesized before the attack of pathogen or infection that is they are present in healthy plants which are not yet infected.
- **Phytochrome:** Phytochromes are a class of photoreceptor in plants, bacteria and fungi used to detect light. They are sensitive to light in the red and far-red region of the visible spectrum
- **Terpenoids:** The terpenoids, also known as isoprenoids, are a large and diverse class of naturally occurring organic chemicals derived from the 5-carbon compound isoprene

10.7 SELF ASSESSMENT QUESTION

10.7.1 Multiple choice questions

1. Primary metabolites are essential for
   (a) Growth (b) Development
   (c) Reproduction (d) All of the above

2. Which of the following is correct function of suberin
   (a) Protection against pathogen (b) Wound healing
   (c) Prevention of wall loss (d) All of the above

3. Jojoba wax is obtained of the following plant
   (a) *Withania somnifera* (b) *Simmon dsiachinensis*
   (c) *Delgerbia indica* (d) *Copernica cerifera*

4. Sesquisterpenes comprises of how many terpene rings
   (a) Four (b) Two
   (c) Three (d) Five

5. Which of the following is NOT CORRECT characteristic feature of terpenoids
   (a) They possess smell or flavor (b) They are water soluble
   (c) They are generally optically active (d) They are oxidizing agent

6. Select the acyclic terpenoids from the following
   (a) Geraniol (b) Menthol
   (c) Thujone (d) Camphor

7. Identify the plant hormone which is a diterpenoid acid and helps in seed germination
   (a) Kinetin (b) Thiodizurane
   (c) Ethylene (d) Gibberellin

8. Sinigrin is a
   (a) O-glycoside (b) S-glycoside
   (c) N-glycoside (d) C-glycosides

9. Secondary glycosides can be derived from primary glycosides by
   (a) Addition of one sugar molecule (b) Removal of one sugar molecule
   (c) Addition of one lipid molecule (d) Removal of one lipid molecule

10. Which of the following is a true alkaloid
   (a) Ephedrine (b) Colchicine
   (c) Pyrrolizidine derivatives (d) Caffeine

11. Select the secondary metabolites with anticancer activity
   (a) Quinone (b) Vincristine
   (c) Morphine (d) Codeine

12. Which of following is NOT true about long day flowering plant (LDP)
   (a) They show best flowering under continuous light
   (b) Alternate period of darkness and light result in flowering
   (c) Flowering is promoted by long period of darkness
   (d) LDP can be made to flower under short day conditions

13. Select short day flowering plant
   (a) Oryza sativa (b) Pisum sativum
   (c) Rye Gras (d) Wheat

14. Choose the correct combination of climacteric fruits
   (a) Papaya and Banana (b) Papaya and Grapes
   (c) Grapes and Banana (d) Pineapple and Lemon

Answer: 1- d, 2- d, 3- b, 4- c, 5- b, 6- a, 7- d, 8- b, 9- b, 10- c, 11- b, 12- c, 13- a, 14- a

10.7.2 State whether the following statements are true or false:

1. Cuticle is composed of three layers outer wax, middle layer of cuticle proper and third cuticular layer.
2. Cutin layer is permeable to water.
3. Presence of suberin in casparian strips restricts entry of microbial pathogens.
4. There is no effective of geographical region on wax production by plant species.
5. Phytoanticipins are high molecular weight compounds with antimicrobial activity.

6. A flash of light during continuous dark period will inhibit flowering in long day flowering plant.
7. Perseverance of stimulus for flowering by leaves was demonstrated by M.K. Chailakhyan.
8. Chrysanthemum is a short-day flowering plant.
9. If all the leaves of plant are defoliated it will foil to flower.
10. During day time Pfr is converted into Pr.

Answers: 1. True, 2- False, 3- True, 4- False, 5- False, 6- False, 7- True, 9- True, 10- False

10.7.3 Fill up the blanks in the following statements:

1. _____ is the protective covering over surface of plant organs.
2. Candelilla wax is obtained from _____ of candelilla.
3. _____ in barley provides protection against *Fusarium sp.*
4. _____ in potato provides protection against *Botrytis cineria*.
5. The term photoperiodism was given by _____ and _____.
6. _____ enzyme is responsible for hydrolysis of pectin bonds.
7. The hormone responsible for ripening of fruits is _____.
8. Orange colour of ripened fruit is mainly due to pigment _____.
9. Non sugar port of glycoside is known as _____.
10. _____ is a common intermediate in biosynthesis of phenolic compounds.

Answer: 1- Cuticle, 2- Leaves, 3- Proanthocynidins, 4- Benzaldehyde, 5- Garver and Allard, 6- Poly galactouronase, 7- Ethylene, 8- Carotenoid, 9- Aglycon/genie, 10- Phenylalanine

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- Panday and Sinha. Plant Physiology.
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10.9 *SUGGESTED READINGS*

- V K Jain. Fundamentals of Plant Physiology
- William G. Hopkins, Norman P. A. Hüner. Introduction to Plant Physiology

10.10 TERMINAL QUESTIONS

10.10.1 Very short answer type questions:

1. Define climacteric fruits with example?
2. What do you understand by the term phytochrome?
3. What are Pseudo alkaloids?
4. Name major chemical constituents of wax?
5. Mention the significance of cutin layer?
6. What are Phytoanticipins?
7. Give two examples of long-day and short-day flowering plants?
8. What are secondary metabolites?

10.10.2 Short answer type questions:

1. Differentiate between climactic and non-climactic fruits?
2. How can long day flowering plants be made to flower under short day conditions?
3. Enlist adverse effects of hormone ethylene?
4. Mention about hormonal control of fruits ripening?
5. Mention about the change in flavor and pigmentation during fruit ripening?
6. Differentiate between qualitative and quantitative short day and long day flowering plants.
7. Briefly mention about day neutral plants?
8. Mention functions of alkaloids
9. Classify glycosides based upon the linkage present. Give example for each class?

10.10.3 Long answer type questions:

1. Describe in detail differences in flowering pattern among short-day and long-day flowering plants?
2. Elaborate about the physiological and biochemical changes occurring during fruit ripening.
3. Analyze effect of phytochrome on flowering in plants?
4. Enlist characteristic features and functions of terpenes?
5. Cite an experimental study conducted to prove that flowering stimulus is perceived by leaves?
6. Elaborate about phenolic compounds as important plant secondary metabolites.
7. Classify terpenoids along with their functions?

UNIT-11-TYPES OF STRESSES, STRESS TOLERANCE AND CONTROL

Contents:

11.1 OBJECTIVES

After reading this unit students will be able

- To understand the different types of stresses encountered by plant i.e. Temperature stresses, water stresses, salt and heavy metal stresses
- To know the effects of different abiotic stresses on plant's growth and development
- To explain the mechanism of abiotic stress response or How plant tolerate stress and
- To know about the stress control.

11.2 INTRODUCTION

Stress in plants refers to any external unfavorable conditions or substance that adversely affects or blocks metabolism, growth, development or productivity of plants. The study of functioning of plants under these stresses or adverse environmental conditions is called as stress physiology. Stresses trigger a wide range of plant responses like altered gene expression, cellular metabolism, changes in growth rates, crop yields, etc. A plant stress usually reflects some sudden changes in environmental condition. The concept of stress is nearly associated with the plant's ability to cope with adverse condition i.e. stress tolerance. If tolerance of a plant increases as a result of exposure to prior stress, the plant is said to be acclimated whereas resistance acquired by the plant through a process of selection over several generations. However in stress tolerant plant species, exposure to a particular stress leads to acclimation to that specific stress in a time-dependent manner. Abiotic stress imposed on plants by environment may be either physical or chemical. The plants can be recovered from injuries if the stress is mild or of short term as the effect is temporary while as severe stresses leads to death of crop plants by preventing flowering, seed formation and induce senescence.

11.3 TYPES OF STRESSES

Plants are subjected to a wide range of environmental stresses that reduces and limits the productivity of plants. Two types of environmental stresses are encountered to plants which can be categorized as (1) Abiotic stress and (2) Biotic stress. The abiotic stress causes the loss of major crop plants worldwide and includes high temperature, cold, drought, floods, salinity and heavy metals. On the other hand, attacks by various pathogens such as fungi, bacteria, nematodes and herbivores are included in biotic stresses. Abiotic stress has been becoming a major threat to food security due to the constant changes of climate and deterioration of environment caused by human activity. This unit includes different types of abiotic stresses encountered by plants.

11.3 TEMPERATURE STRESSES

Temperature is one of the major environmental factors which affect plant growth, development, and yield. Temperatures persistently above optimal and below optimal have

adverse effects for plant growth which ultimately reduce yields. Temperature stress above optimal is Heat stress (high temperature stress) whereas below optimal it is Cold Stress (low temperature stresses). Temperature stress results in low germination rates, growth retardation, reduced photosynthesis, and at some threshold, they will be lethal.

11.4.1 Heat stress (High temperature stress)

Heat stress is often defined as the rise in temperature beyond a threshold level for a period of time sufficient to cause irreversible damage to plant growth and development. In general, a transient elevation in temperature, usually 10-15°C above ambient, is considered heat shock or heat stress. Heat stress has negative effects on crop physiology (Fig.11.1) (e.g. decreased photosynthesis, increased respiration). Another adverse effect of heat stress is the negative influence on the plant root system, which provides support, nutrient and water uptake, and transport to other plant organs (Valdés-López et al., 2016), resulting in disrupted pollination, flowering, root development, and root growth stages (Sehgal et al., 2017; Cho, 2018). Heat stress also disturbs cellular homeostasis and causes denaturation and dysfunction in numerous proteins. It also alters the efficiency of enzymatic reactions in the cell and creates metabolic imbalance. The response to a sudden increase in temperature of 5-10^{o}C, makes plants produce a unique protein called "heat-shock proteins" (HSP) or "stress-induced proteins" or "stress proteins. HSPs were originally discovered in fruit fly (*Drosophila melanogaster*). Increased HSP production occurs when plants experience an increase in temperature either suddenly or gradually. Under these conditions, HSP is useful for protecting proteins. The majority of HSPs are molecular chaperones and play an important role in protein stabilization such as assembling of multi-protein complexes, folding or unfolding of proteins, transport or sorting of proteins into correct compartments at sub-cellular level, control of cell-cycle and signaling, as well as cell protection against stress or apoptosis. HSPs prevent accumulation of proteins with anomalous conformations and eliminate non-native aggregations formed during stress, with ubiquitin-mediated degradation of these proteins. The induction and synthesis of heat-shock proteins due to high temperature exposure are common phenomena in all living organisms from bacteria to human beings. Hsps are classified into five principal classes according to their approximate molecular weight: (1) Hsp100, (2) Hsp90, (3) Hsp70, (4) Hsp60, and (5) small heat-shock proteins (sHsps).

In plants, the major sites of heat stress injury are the oxygen-evolving complex (OEC) along with linked biochemical reactions in photosystem II (PSII). Perception of heat stress by plants usually triggers sensors at the plasma membrane and causes a transient opening of Ca^{2+} channels, possibly via modulation of membrane fluidity. In fact, the overproduction of reactive oxygen species (ROS) causing wide-ranging cellular damage and inhibition of physiological functions in plants. Nevertheless, stress positively leads to induce Ca^{2+} influx and cytoskeletal restructuring, ensuing in the upregulation of mitogen activated protein kinases (MAPK) and calcium dependent protein kinase (CDPK). Signaling of these cascades at nuclear level that leads to the production of antioxidants and compatible osmolytes for cell water balance and osmotic adjustment. The

sensing of high temperature and induction of signaling cascades are vital adaptive ladder in managing with challengers of heat stress. Significantly, thermotolerance mechanism is largely connected to display of heat shock response and this is completed by reprogramming of gene expression; thereby allowing plants to manage with heat stress. For that reason, greater emphasis on heat stress management is obligatory for heat tolerance features including the maintenance of cell membrane stability, capturing the reactive oxygen species (ROS), synthesis of antioxidants, accumulation and osmoregulation of osmoticum, induction of some kinases that respond to stress, Ca-dependent kinase proteins, and enhancing the transcription and signal transfer of chaperones (Wahid et al., 2007).

Fig.11.1: Major effects of heat stress on plants.

11.4.1 Cold stress (Low temperature stress)

The temperatures considerably lower than the optimal growth temperatures i.e. below 10-15°C result in two temperature stress in plant. This is decided by the fact whether there is formation of ice within the tissues or not. Both these condition also cause water deficit inside the plant. These two types of low temperature stresses are: Chilling stress and freezing stress. The chilling low-temperature stress occurs when temperatures are lowered to below 10-15°C, whereas the freezing low-temperature stress occurs when temperatures are lowered to below 0°C and ice forms within tissues. Low temperatures can damage plants both by a chilling, leading to physiological and developmental abnormalities, and by freezing, causing cellular damage directly or via cellular dehydration resulting in decreased chlorophyll content, photosynthetic rate, stomatal conductance, transpiration rate, and electron transport rate (ETR) (Fig. 2). Plants respond differently to low temperatures and based on this they can be again differentiated as;

Chilling sensitive plants: These plants are seriously injured by temperature above 0°C, below 15°C. Most plants of tropical and subtropical origin belong to this group.

Chilling resistant Plants: These plants are able to tolerate chilling temperatures and are seriously injured when ice start to form in tissues. The ratio of unsaturated to saturated fatty acids is higher in chilling resistant plants.

Frost Resistant Plants: These plants tolerate exposure to very low temperatures (-50°C to -100°C) even when immersed in liquid N2.

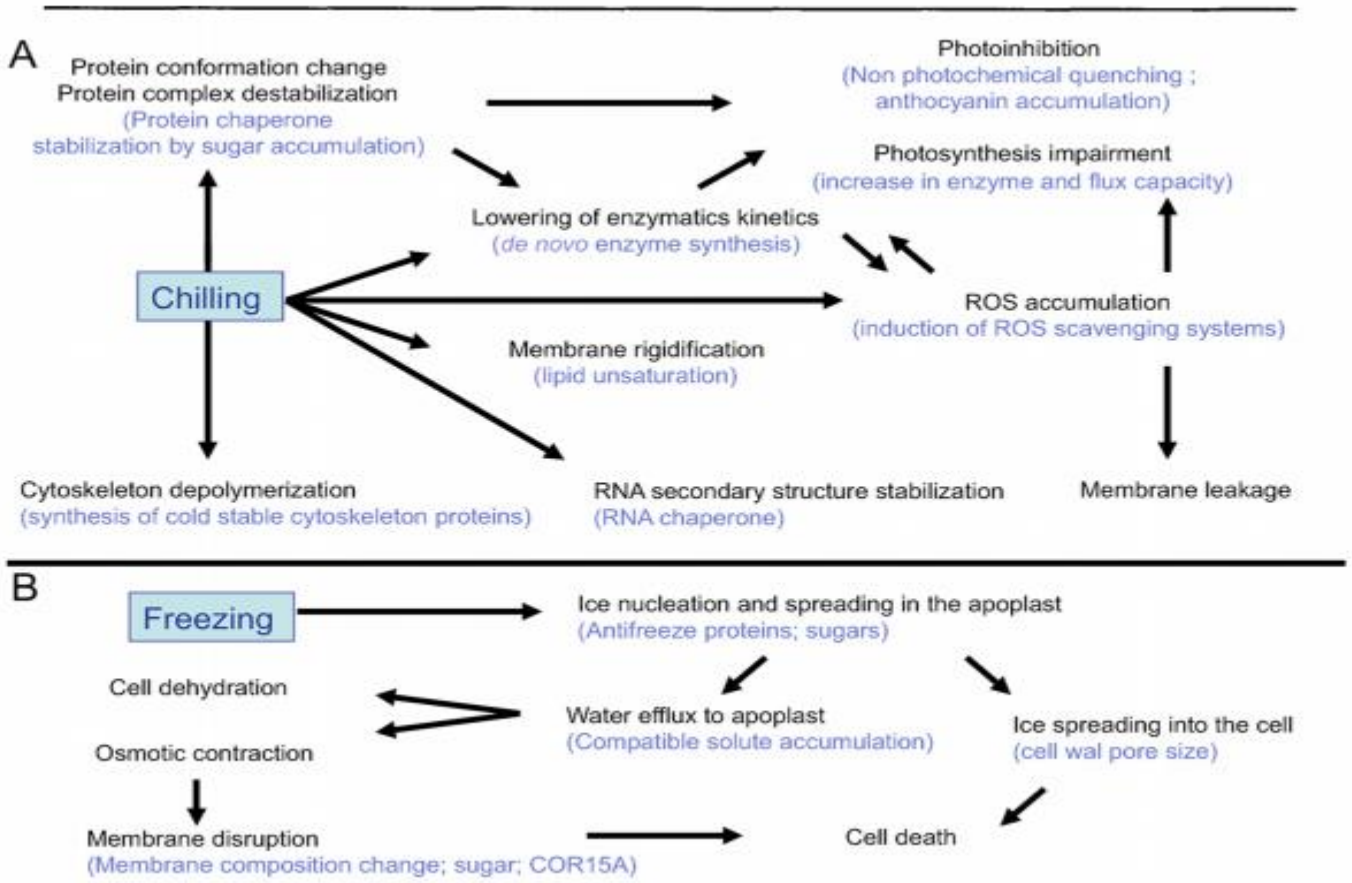

Fig11.2: Effect of chilling and freezing on cellular processes and the cellular responses to chilling (in parentheses) that will lead to chilling tolerance and freezing tolerance (Ruelland et al., 2009)

Chilling stress: Chilling stress or injury leads to a rapid wilting of the leaves and the development of water soaked patches that go on to form sunken pits due to cell collapse. Warming will lead to these damaged areas becoming brown and necrotic while continued chilling will eventually lead to the death of the plant. Plants may develop physiological disorders when exposed to low but non-freezing temperatures. Changes in membrane structure and composition, decreased protoplasmic streaming, electrolyte leakage and plasmolysis i.e. cellular changes occurs. Increased or reduced respiration, depending on severity of stress, production of abnormal metabolites due to anaerobic condition i.e. metabolic changes.

Freezing stress: Freezing stress or injury in plants can be from two sources i.e.; Freezing of soil water, and freezing of the fluids within the plant. The soil water that is available to plants is found in the porous regions between soil particles. It freezes at about -2°C, depriving the plant of its source of water. Freezing of water within the plant is a more serious threat, as it can cause disruption of structure and function of cells and tissues. Freezing damage occurs primarily due to the formation of ice crystals, which damage cell structure when the temperature falls below 0°C. Ice usually forms first in the cell walls and intercellular spaces and damage occurs when ice crystals grow and puncture into the cytoplasm. In plant cells and tissues two types of freezing takes place;

(i) Vitrification: Solidification of the cellular content into non-crystalline state (amorphous state). It occurs by rapid freezing of cells (decrease in temperature by more than 3°C / min) to a very low temp.

(ii) Crystallization / ice formation : Crystallization of ice occur either extracellularly or intracellularly (gradual cooling /drop in temperature)

11.5 WATER STRESS

Nowadays climate has changed all around the globe by continuously increase in temperature and atmospheric CO_2 levels. This change in climate has resulted in uneven distribution of rainfall which acts as water stress as either **drought** i.e. deficiency of water or as **flooding** i.e. excess of water. Plant growth and productivity are adversely affected by water stress. In fact, plants have evolved various molecular mechanisms to reduce their consumption of resources and adjust their growth to adapt to adverse environmental conditions.

11.5.1 Drought

Drought is a meteorological term and is commonly defined as a period without significant rainfall. Generally drought stress occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration or evaporation. Dought is a meteorological term and is commonly defined as a period without significant rainfall. Generally drought stress occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration or evaporation. Drought is a meteorological term and is commonly defined as a period without significant rainfall. Generally drought stress occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration or evaporation.

Drought is a meteorological term and is commonly defined as a period without significant rainfall. Generally drought stress occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration or evaporation. Principally, drought stress occurs when the soil water potential falls between -0.5 and -1.5 MPa. Drought events limit plant performances in different developmental stages. Limited water availability can indeed reduce the germination rate and the development of young plants. During the progression of plant growth, drought basically influences the plant water relations, which in turn cause severe perturbation to the whole plant metabolism (at physiological, biochemical and molecular levels), depending to the stress severity and duration.

Water deficit conditions alter several activities of plant, but one of the main effects is the decline of photosynthetic activity through photo-oxidation and enzyme damage, thereby decreasing the amount of assimilates available for export to the sink organs and finally the plant yield. During such conditions, oxidative stress, directly or indirectly generated in plants, is one of the main drivers of plant responses and results in damage to cell membrane, altering membrane integrity, physiological and biochemical alterations which lead to acute metabolic disorders and eventually alter the plant productivity. Besides this, carbohydrate metabolism in plants is severely altered, ultimately affecting both biological and economical yield. After drought is imposed on crop plants growth arrest is the first response subjected on the plants. Plants reduce their growth of shoots under drought conditions and reduce their metabolic demands. After that protective compounds like abscisic acid (ABA), proline, mannitol, sorbitol, radical scavenging compounds (ascorbate, glutathione, α-tocopherol etc.), and new proteins and mRNAs are synthesized by plants under drought by mobilizing metabolites required for their osmotic adjustment.

Plants under drought conditions use various changes to tolerate stress conditions and increase drought tolerance which includes changes in whole-plant, tissue, at physiological and molecular levels. Plants use various morphological mechanisms operative under drought conditions like: Drought escape or drought avoidance. Escape from drought allowed production of new seeds before the harsh environment conditions end the life cycle of the plant. In these conditions, plants develop rapidly and reduce vegetative growth period. Also, early flowering is an important mechanism plant use to adapt with drought. Therefore, short life cycle considered a proper technique to escape from climatic stresses. Whereas, avoidance has been referred to as dehydration avoidance, avoidance mechanisms and depends on the decrease water loss from plants by control transpiration, and increase water use efficiency. At the same time, the root system plays a vital role in avoiding drought mechanism and the root system characters change (it becomes deeper and thicker) to adsorbing water from extra depths to contribute to producing yield under drought conditions. Drought tolerance characters studied are primarily involved with protection of cellular structure from the effect of cellular dehydration. Dehydrins and late-embryogenesis abundant (LEA) proteins are being accumulated in response to decrease in plant tissue water content. These proteins are said to act as chaperones that protect protein and membrane structure. Compatible solutes can also protect protein and membrane structure under dehydration. The role of reactive oxygen species (ROS) in stress signaling have been extensively studied in recent years.

11.5.2 Flooding stress

Flooding stress in terrestrial species is referred to as either waterlogging or submergence depending on the depth of the water table. In a broad sense, the term flooding is often used to depict different situations in which the water excess can range from water saturated soil (i.e. waterlogging) to deep water columns causing complete submergence of plants (fig.3). Waterlogging corresponds to the full saturation of the soil pores with water, and with a very thin-or even without a layer of water above the soil surface. Hence, under waterlogged

conditions, only the root system of plant is under the anaerobic conditions imposed by the lack of oxygen, while the shoot is under atmospheric normal conditions. Flooding is the situation in which there is a water layer above the soil surface. This water layer can be shallow or deep, so that it can provoke partial or complete submergence of plants. Under partial submergence conditions, plants have a portion of their shoots underwater, besides having their roots completely immersed in water-saturated soil. Under complete submergence, plants confront the most stressful scenario because both, shoot and root plant compartments are underwater.

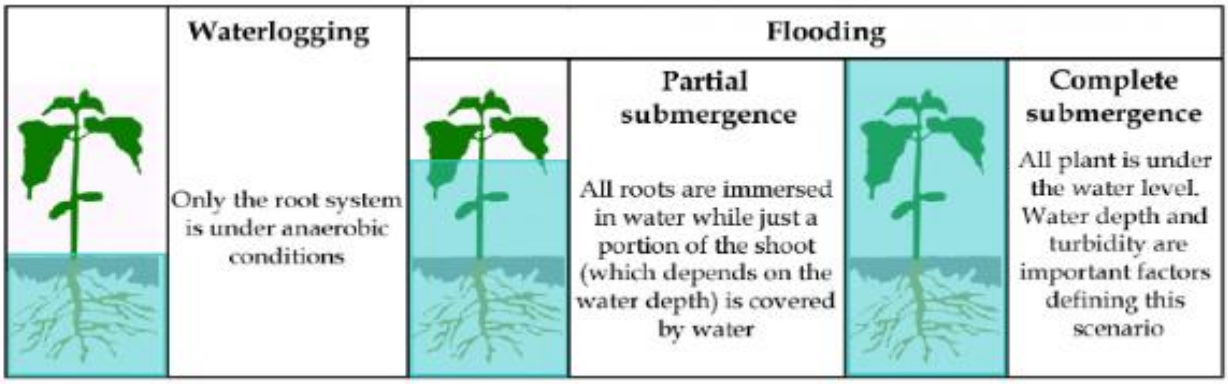

Fig. 11.3. *Scheme of the different scenarios encountered by plants in front to increasing levels of water excess, ranging from waterlogging to complete submergence.*

Soil flooding creates composite and complex stress in plants and the damage symptoms caused are primarily due to the prolonged exposure of the plants to hypoxia. The effect of waterlogging of roots and lower stems are apparent as a range of symptoms on the shoots, including rapid wilting and severe physiological disruption. As flooding time increases, a second problem associated with water excess appears as a result of the progressive decrease in the soil reduction-oxidation potential. With the reduction of the soil redox potential potentially toxic compounds appear such as sulfides, soluble Fe and Mn, ethanol, lactic acid, acetaldehyde and acetic and formic acid. Therefore, the lack of oxygen and later the accumulation of some potentially toxic compounds are the major constraints that plants suffer under flooding conditions. Vast areas of rainfed crops, particularly in South and Southeast Asia, are annually affected by flooding. In nature, these stresses are important factors dictating the species composition of the ecosystem. On agricultural land, they cause economic damage associated with long-term social consequences.

11.6 SALINITY STRESS

Salinity is a major stress limiting the increase in the demand for food crops. More than 20% of cultivated land worldwide (~ about 45 hectares) is affected by salt stress and the amount is increasing day by day. Plants on the basis of adaptive evolution can be classified roughly into two major types: the halophytes (that can withstand salinity) and the glycophytes (that cannot withstand salinity and eventually die). Majority of major crop species belong to this second category. Thus salinity is one of the most brutal environmental stresses that hamper crop productivity worldwide and is defined as the presence of excessive amounts of soluble salts that

hinder or affect the normal functions of plant growth. Salinity stress involves changes in various physiological and metabolic processes, depending on severity and duration of the stress. It is measured in terms of electrical conductivity (ECe), with the exchangeable sodium percentage (ESP) or sodium adsorption ratio (SAR) and pH of a saturated soil paste extract. Therefore, saline soils are those that have saturated soil paste extracts with an ECe of more than 4 dSm–1 , ESP less than 15 percent, and pH below 8.5. Saline soils have a mixture of salts of Chloride, Sulfate, Sodium, Magnesium and Calcium ions with sodium chloride. Most plants cannot survive when NaCl concentrations exceed 200 mM (Zhou J.C. et al., 2016) because high salinity extensively impinges on their lifecycle comprising seed germination, seedling establishment, vegetative growth, and flower fertility (Guo et al., 2018). Two primary effects are imposed on crop plants by salt stress; first is osmotic stress and second ion toxicity. Initially soil salinity is known to represses plant growth in the form of osmotic stress which is then followed by ion toxicity.

During the initial phases of salinity stress, water absorption capacity of root systems decreases and water loss from leaves is accelerated due to osmotic stress of high salt accumulation in soil and plants, and therefore salinity stress is also considered as hyperosmotic stress. Osmotic stress in the initial stage of salinity stress causes various physiological changes, such as interruption of membranes, nutrient imbalance, impairs the ability to detoxify reactive oxygen species (ROS), differences in the antioxidant enzymes and decreased photosynthetic activity, and decrease in stomatal aperture. Salinity stress is also considered as a hyperionic stress. One of the most detrimental effects of salinity stress is the accumulation of Na^+ and Cl^- ions in tissues of plants exposed to soils with high NaCl concentrations. Entry of both Na^+ and Cl^- into the cells causes severe ion imbalance and excess uptake might cause significant physiological disorder(s). High Na^+ concentration inhibits uptake of K^+ ions which is an essential element for growth and development that results into lower productivity and may even lead to death. In response to salinity stress, the production of ROS, such as singlet oxygen, superoxide, hydroxyl radical, and hydrogen peroxide, is enhanced. Salinity-induced ROS formation can lead to oxidative damages in various cellular components such as proteins, lipids, and DNA, interrupting vital cellular functions of plants. Genetic variations in salt tolerance exist, and the degree of salt tolerance varies with plant species and varieties within a species. Salt stress reduces growth of crops and yield in many ways, as it is interlinked with drought, which is another global issue, and that is aggravated by extreme temperatures (Slama et al., 2015).

11.7 METAL STRESS

Many heavy metals occur naturally in the earth's crust at various levels but the problem arises when they are released in excess into the environment due to natural and/or anthropogenic activities. During the last few decades, increased anthropogenic activities such as use of pesticides, fertilizers, municipal and compost wastes, heavy metal release from smelting industries and metalliferous mines have contaminated large areas of land. During evolution of angiosperms, only 19 elements such as C, O, H, Mg, S, N, Ca, P, and K (macronutrients) and Cu,

Zn, Mn, Fe, Mo, B, Ni, Co, Cl, and B (micronutrients) were selected for basic metabolism. In addition, Si is also considered as a beneficial element, and it has been reported to be involved in the maintenance of plant structures in some plants (Epstein, 1999). Macro and micronutrients play an important role in physiological and biochemical processes of plants such as chlorophyll biosynthesis, photosynthesis, DNA synthesis, protein modifications, redox reactions in the chloroplast and the mitochondrion, sugar metabolism, and nitrogen fixation. For example, Zn is a cofactor for more than 300 enzymes and 200 transcription factors associated with the maintenance of membrane integrity, auxin metabolism, and reproduction (Ricachenevsky et al., 2013). However, at elevated concentrations, heavy metals produce severe toxicity symptoms in plants, and therefore, their uptake and utilization are tightly controlled by the plant cells. Some heavy metals, such as Cd, Cr, Pb, Al, Hg, etc., although being non-essential and without physiological function, are very toxic even at very low concentrations (Gill et al., 2013).

Heavy metals whether essential or nonessential generally produce common toxic effects on plants, such as low biomass accumulation, chlorosis, inhibition of growth and photosynthesis, altered water balance and nutrient assimilation, and senescence, which ultimately cause plant death. Plants employ various inherent and extrinsic defense strategies for tolerance or detoxification whenever confronted with the stressful condition caused by the high concentrations of heavy metals. As a first step towards dealing with metal intoxication, plants adopt avoidance strategy to preclude the onset of stress via restricting metal uptake from soil or excluding it, preventing metal entry into plant root. This can be achieved by some mechanisms such as immobilization of metals by mycorrhizal association, metal sequestration, or complexation by exuding organic compounds from root. At next stage, if these strategies fail and heavy metals manage to enter inside plant tissues, tolerance mechanisms for detoxification are activated which include metal sequestration and compartmentalization in various intracellular compartments (e.g., vacuole), metal ions trafficking, metal binding to cell wall, biosynthesis or accumulation of osmolytes and osmoprotectants, for example, proline, intracellular complexation or chelation of metal ions by releasing several substances, for example, organic acids, polysaccharides, phytochelatins, and metallothioneins, and eventually if all these measures prove futile and plants become overwhelmed with toxicity of heavy metal, activation of antioxidant defense mechanisms is pursued.

The functional diversity and molecular versatility of phytochelatins (PCs) and metallothioneins (MTs) are becoming intriguing when it comes to heavy metal detoxifcation and maintaining cellular ion balance. Their role seems to go beyond being mere heavy metal-chelating peptides or heavy metal vacuolar and cellular sequestrators. They may act as cellular homeostatic or detoxifying agents. PCs and MTs are likely to interact directly or indirectly with plant antioxidant defense system or get involved in translocating and distributing excessive ion metals between root and shoot in a timeor tissue-specifc manner. There are also obvious indications that use of transgenic plants over expressing PCs or confer substantial heavy metal tolerance. Therefore, transgenic and candidate gene approaches can be effectively adopted for

phytoremediation purposes or for the fortification of plants that are deficient in PCs or MTs (Emamverdian et al. 2015).

11.8 STRESS TOLERANCE AND CONTROL

As plants are sessile in nature, they have no choice to escape from these environmental cues. Plants have developed various mechanisms in order to overcome these threats of stresses. They sense the external stress environment, get stimulated and then generate appropriate cellular responses. Tolerance in plants is a complex trait, involving many different metabolic pathways and cellular and molecular components. The ability of the plant to cope with or adjust to the stresses varies across and within species as well as at different developmental stages. They do this by stimuli received from the sensors located on the cell surface or cytoplasm that can initiate a number of molecular, cellular, and physiological changes to respond and adapt to such stresses. Below given is the figure (Fig. 11.4) that shows different defense responses exhibited by plant in order to tolerate the different stresses.

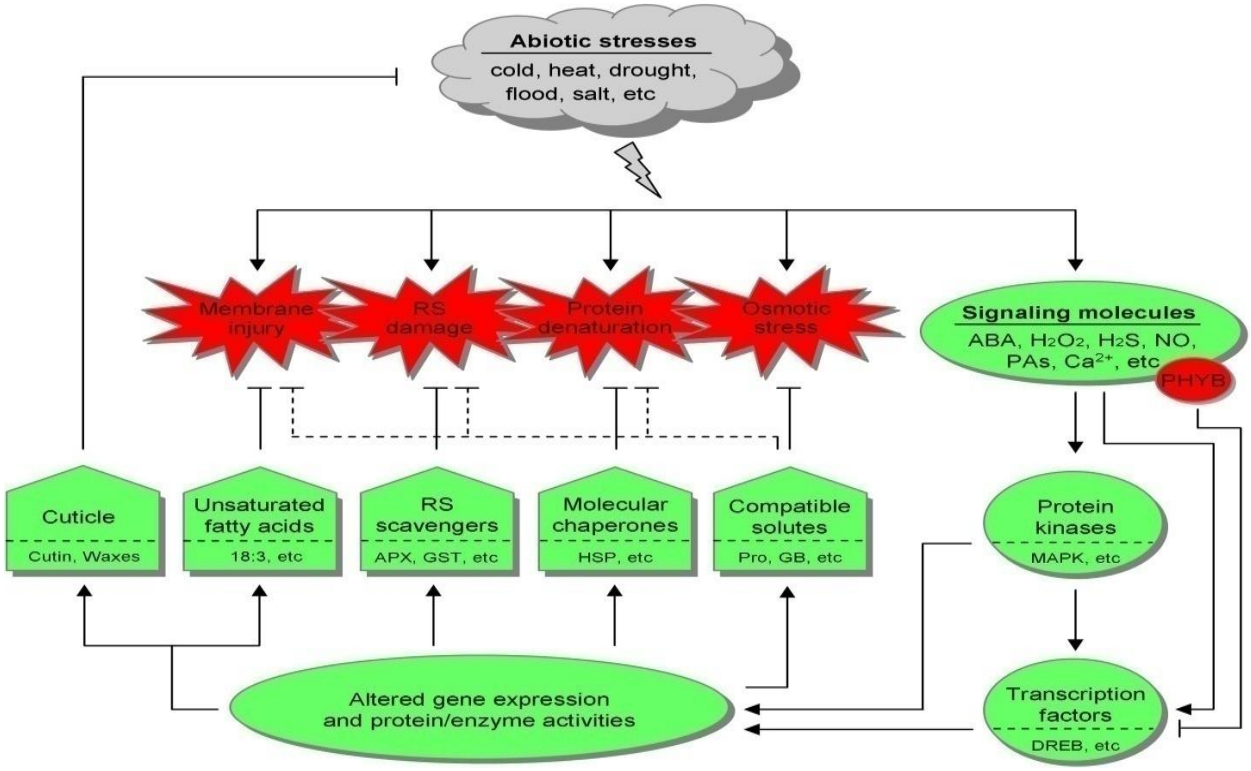

Fig. 11.4: *The general defense system and the underlying regulatory network in responses to abiotic stresses.*

Different abiotic stresses, such as cold, heat, drought, flood, and salt can provoke common cellular disorder and secondary stresses, including membrane injury, reactive species (RS) damage, protein denaturation, and osmotic stress, which are also interconnected with each other. Accordingly, land plants have resorted to unsaturated fatty acids, RS scavengers, molecular chaperones, and compatible solutes. Some compatible solutes may also be involved in

counteracting other adverse effects, as indicated with dotted inhibitory lines. Besides, the cuticle serves as the universal outermost shield. Upon stress stimulation, signaling molecules mobilize the downstream effectors, primarily protein kinases and transcription factors, leading to altered gene expression and protein/enzyme activities, thereby launching the defense systems. Notably, phytochrome B (PHYB) is emerging as a negative regulator in stress tolerance.
Where, 18:3-linolenic acid; APX-ascorbate peroxidase; GST-glutathione *S*-transferase; HSP-heat shock protein; Pro-proline; GB-glycine betaine; ABA-abscisic acid; PAs-polyamines; MAPK-mitogen-activated protein kinase; DREB-dehydration responsive element binding factor.

Plants cope with adverse temperature stress by altering molecular mechanisms involving proteins, antioxidants, metabolites, regulatory factors, other protectants and membrane lipids. Thermotolerance is closely correlated with the production of toxic acrolein and methyl vinyl ketone from membrane trienoic fatty acids under heat stress, and it is possible to produce thermotolerant plants with reduced trienoic fatty acid contents. Regulatory factors such as heat shock factors directly and/or indirectly induce accumulation of stress-related gene products under high temperature conditions and contribute to thermotolerance. Under high temperature conditions, several protectants, such as glycinebetaine which apparently stabilizes photosystem II proteins, accumulate to protect proteins and photosystems in plants. Antioxidants decrease levels of stress-inducible reactive oxygen species, contribute to improved tolerance to cold as well as high temperature stress. To counter the cold stress, the plants resort to diverse defense mechanisms. Various studies on cold stress report manifold rise in cellular levels of osmolytes such as proline, glycine betaine, sugars such as trehalose, fructans, and sugar alcohols, which have a pivotal role in osmotic adjustment during water-deficit situation. Under cold stress, these molecules may also serve as cryoprotectants to save the cellular metabolism by protecting the integrity of membranes and cellular organelles, maintaining the redox potential and components of vital pathways, saving the photosynthetic machinery and also acting as partial antioxidants. The new roles of cryoprotectants are emerging under cold stress making them candidates for genetic manipulation to improve cold tolerance. Some proteins are also synthesized during acclimation of plant to freezing which are called as antifreeze proteins. These proteins are believed to stabilize other proteins and cell membranes during dehydration of cells induced by cold temperatures.
Many plants have improved their resistance mechanisms to tolerate drought stress, but these mechanisms are varied and depend on the plant species. Typically, mechanisms involved in plant tolerance to drought follow a general plan: maintaining cell homeostasis in water-deficit situations, which is possible by increasing the water inlet to the cells. Drought avoidance is other common drought resistance mechanism in annual plants. With this mechanism, escape from stress conditions is the main strategy for plant growth under drought conditions whereas aerenchyma formation in the root cortex is one of the main response to flooding or partial submergence tolerance. This aerenchymatic tissue provides a continuous system of interconnected aerial spaces of lower resistance for oxygen transport from aerial shoots to

submerged roots, allowing root growth and soil exploration under anaerobic conditions. In roots, the formation of adventitious roots is highlighted as a common response of flood-tolerant species. These adventitious roots, which have high porosity, help plants to continue with water and nutrient uptake under flooding conditions.

In order to survive in soils with high salt concentration plants develop various physiological and biochemical mechanisms. Principle mechanisms include, but are not limited to, (1) ion homeostasis and compartmentalization, (2) ion transport and uptake, (3) biosynthesis of osmoprotectants and compatible solutes, (4) activation of antioxidant enzyme and synthesis of antioxidant compounds, (5) synthesis of polyamines, (6) generation of nitric oxide (NO), and (7) hormone modulation. Increasing evidence demonstrates the roles of a Salt Overly Sensitive (SOS) stress signalling pathway in ion homeostasis and salt tolerance. The SOS signalling pathway consists of three major proteins, SOS1, SOS2, and SOS3. SOS1, which encodes a plasma membrane Na^+/H^+ antiporter, is essential in regulating Na^+ efflux at cellular level. It also facilitates long distance transport of Na^+ from root to shoot. Over-expression of this protein confers salt tolerance in plants.

Plants also possess a sophisticated and interrelated network of defense strategies to tolerate heavy metal intoxication. Some morphological structures like thick cuticle, biologically active tissues like trichomes, and cell walls as well as mycorrhizal symbiosis can act as barriers when plants are faced with heavy metal stress. Biosynthesis of diverse cellular biomolecules is the primary way to tolerate or neutralize metal toxicity. This includes the induction of a myriad of low-molecular weight protein metallochaperones or chelators such as nicotianamine, putrescine, spermine, mugineic acids, organic acids, glutathione, phytochelatins, and metallothioneins or cellular exudates such as flavonoid and phenolic compounds, protons, heat shock proteins, and specific amino acids, such as proline and histidine, and hormones such as salicylic acid, jasmonic acid, and ethylene. When the above-mentioned strategies are not able to restrain metal poisoning, equilibrium of cellular redox systems in plants is upset, leading to the increased induction of ROS.

To mitigate the harmful effects of free radicals, plant cells have developed antioxidant defense mechanism which is composed of enzymatic antioxidants like superoxide dismutase (SOD), catalase, (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and glutathione reductase (GR) and nonenzymatic antioxidants like ascorbate (AsA), glutathione (GSH), carotenoids, alkaloids, tocopherols, proline, and phenolic compounds (flavonoids, tannins, and lignin) that act as the scavengers of free radicals. Plant-associated microbes could be used as an alternate strategy for sustainable agricultural production. Numerous plant-associated microbes namely, bacteria and fungi are known to exhibit plant-growth promoting traits under heavy metal stress. These microbes impart favourable effects on plants via several direct and indirect mechanisms such as biofilm formation, siderophores, exopolysaccharide, and phytohormones production (Tiwari et al., 2017b). Since microbial heavy metal remediation does not involve any transgenic modifications, it is ethically and societally acceptable.

11.9 CHEMICAL MODULATIONS

Abiotic stresses disturb plant growth and yield formation. Several chemical compounds, known as plant growth regulators (PGRs), modulate plant responses to biotic and abiotic stresses at the cellular, tissue, and organ levels. Chemical treatments of chilling-sensitive plants lead to increased chilling tolerance.

- **Thiourea (TU):** It is an important synthetic PGR containing nitrogen (36%) and sulfur (42%) that has gained wide attention for its role in plant stress tolerance. Tolerance against abiotic stresses is a complex phenomenon involving an array of mechanisms, and TU may modulate several of these. Exogenous application of TU (e.g. as seed priming, foliar spray, medium supplementation, soil application) stimulates defense mechanisms in plants under abiotic stress including photosynthesis, nitrogen metabolism, proline metabolism, antioxidant defense systems, and plant water relations during different plant developmental stages.
- **Dimethyl sulfoxide (DMSO):** A treatment of DMSO, which is a membrane rigidifier, has reported to enhanced the cold acclimation of *Arabidopsis* by activating the MEKK1-MKK2-MPK4 cascade. These results indicate that chemicals modifying lipid fluidity are a possible means of cold adaptation in plants.
- **Sulfur:** It is the constituent of amino acids such as cysteine and methionine, as well as several compounds, for example, vitamins (thiamine and biotin), coenzymes, thioredoxin system, glutathione, lipoic acids, and glucosinolates. Numerous studies have proved that sulfur not only improves plant productivity during favorable conditions but also provides protection under different abiotic stresses such as salinity, drought, excess temperature, high light, and toxic metals or metalloids.
- **Pyrabactin:** Pyrabactin, a synthetic ABA derivatives that mimic ABA; it activates the ABA receptors needed for improving drought tolerance. Unlike natural ABA, Pyrabactin is not sensitive to light, is easy to synthesize, and relatively inexpensive, and its manufacture for agricultural use is therefore practical.
- **Acetic Acid:** The external application of acetate enhances drought tolerance in various plant species, such as *Arabidopsis*, maize, rapeseed, rice, and wheat (Kim et al., 2017). This effect is related to a novel drought tolerance mechanism in plants involving the acetate-jasmonate signaling pathway, which is regulated epigenetically and conserved in plants. Thus, the external application of acetate to crops is potentially a useful, simple, and low-cost method of enhancing drought tolerance in various plant species.
- **Non-hormonal growth regulators:** They are used also in order to improve the chilling tolerance of cultivated plants. These include paclobutrazol, chlorocholinchloride, mefluidid, uniconazol and other triazoles. The treatment by antioxidants and free radicals quenching (ethoxyquin, sodium benzoate, glutathione, tyrone, formate, ascorbate, diphenylamine, α-tocopherol, propyl-gallate) can slow down the degradation of unsaturated fatty acids and reduce chilling damage in chilling-sensitive plants, leaves and fruits.

11.10 BIOCHEMICAL APPROACHES

Any compound that contains carbon and is found in living things is regarded as biochemical compound. There are four classes of biochemical compounds namely carbohydrates, proteins, lipids (fats), and nucleic acids. Carbohydrates are among the first organic compounds formed during photosynthesis therefore acts as primary energy storage compound. Along with proteins, lipids are the most abundant component of membranes and they play a role in the resistance of plant cells to environmental stresses whereas nucleic acid are the genetic material of cell and participate in signaling under stress.

- **Phytohormones:** Plant growth regulators or phytohormones are substances that influence physiological processes of plants at very low concentrations. Both these terms have been used interchangeably, particularly when referring to auxins, gibberellins, cytokinins, ethylene and abscisic acid. They also include brassinosteroids, salicylic acid, and jasmonic acid that have been shown to play key roles in regulating the plant development under low-temperature stress. Under drought, endogenous contents of auxins, gibberellins and cytokinin usually decrease, while those of abscisic acid and ethylene increase. Nevertheless, phytohormones play vital roles in drought tolerance of plants as auxins break root apical dominance helping in new root formation induced by cytokinins. It has been proposed that abscisic acid and cytokinin have opposite roles in drought stress and were most effective of all plant growth regulators. Increase in abscisic acid and decline in cytokinins levels favour stomatal closure and limit water loss through transpiration under water stress. Ethylene determines the onset of natural senescence and mediate drought-induced senescence. Recent studies suggest that growth promotion is a common feature in ethylene responses. Under complete submergence, concentrations of ethylene increase, which downregulates the abscisic acid levels, and upregulates those of gibberellins. Increased gibberellins level promotes the expression of genes encoding cyclins and expansins, associated with cell division and cell extension (respectively), which lead to a fast shoot elongation underwater. Besides, the increase in endogenous ethylene produces a lower pH in the apoplast that favours the action of expansins provoking the cell wall loosening as a necessary step that precedes cell extension. Plants can optimize growth and tolerate abiotic stresses such as drought, and this response also involves ethylene synthesis. Since various stresses induce ABA synthesis, therefore it is now considered as a plant stress hormone. Plants have to adjust ABA levels constantly in response to changing physiological and environmental conditions. The application of ABA to plant mimics the effect of a stress condition. As many abiotic stresses ultimately results in desiccation of the cell and osmotic imbalance, there is an overlap in the expression pattern of stress genes after cold, drought, high salt or ABA application. This suggests that various stress signals and ABA share common elements in the signaling pathway and these common elements cross-talk with each other, to maintain cellular homeostasis. Some other compounds having hormonal properties, such as salicylic acid and brassinosteroids, also participate in plant abiotic stress responses. Salicylic acid is known to play a critical role in defense responses mainly under biotic stress but it has also

showed an evident role under various abiotic stress such as in thermotolerance and in the oxidative damage generated by NaCl and osmotic stress in Arabidopsis seedlings (Borsani et al.,2001). Better understanding of these events and genes controlling these events could open new strategies for improving tolerance mediated by phytohormones.

- **Osmolytes:** Osmolytes such as proline, glycine betaine, sugars such as trehalose, fructans, and sugar alcohols, plays a pivotal role in osmotic adjustment during water-deficit situation. Many studies on cold stress have reported manifold rise in cellular levels of osmolytes. Under cold stress, these molecules may also serve as cryoprotectants to save the cellular metabolism by protecting the integrity of membranes and cellular organelles, maintaining the redox potential and components of vital pathways, saving the photosynthetic machinery and also acting as partial antioxidants. Their increase in synthesis in the cell could participate in an increase in heat tolerance (Alia et al., 1998). Glycine betaine is a major nontoxic cellular osmolyte that that accumulates in various plant species in response to stresses such as drought and salinity. It raises the osmolarity of the cell during stress period; thus it plays an important function in stress mitigation. Glycine betaine also protects the cell by osmotic adjustment, stabilizes proteins, and protects the photosynthetic apparatus from stress damages and reduction of ROS. Its role as a biostimulant has been subjected to field tests, and it is already being produced commercially. Intracellular proline which is accumulated during salinity stress not only provides tolerance towards stress but also serves as an organic nitrogen reserve during stress recovery.
- **Polyamines:** Polyamines, a group of small aliphatic amines, are reported to have profound influence on plant growth and development. Being cationic, polyamines can associate with anionic components of the membrane by stabilizing and maintaining cellular ionic balance, such as phospholipids, thereby protecting the lipid bilayer from deteriorating effects of stress. Polyamines are also known to accumulate under salt stress conditions in different plant systems, resulting in presumed protective effects, acting as free radical scavengers under these conditions.
- **Lipids:** Increasing the degree of unsaturation of fatty acids leads to an increase in cold tolerance. The opposite situation is that increasing the degree of saturation could lead to heat tolerance (Grover et al., 2000).
- **Carbohydrates:** Accumulations of carbohydrates such as sugars (e.g., glucose, fructose, fructans, and trehalose) and starch occur under salt stress. The major role played by these carbohydrates in stress mitigation involves osmoprotection, carbon storage, and scavenging of reactive oxygen species. Sugar alcohols are a class of polyols functioning as compatible solutes, as low molecular weight chaperones, and as ROS scavenging compounds. They can be classified into two major types, cyclic (e.g., pinitol) and acyclic (e.g., mannitol).
- **Nitrophenolates:** Nitrophenolates are biostimulants and are already being manufactured commercially in Japan under the name Atonik, a synthetic product composed of three phenolic compounds: sodium *p*-nitrophenolate (0.3%), sodium *o*-nitrophenolate (0.2%), and sodium 5-nitroguaiacolate (0.1%), together with water. Atonik has been used successfully for many years in the cultivation of most globally important crops. Its mode of action is still not

understood but might be involved in hormone regulation, nutrient uptake, and nitrogen metabolism (Przybysz et al., 2014). Atonik therefore stimulates plant growth and development and contributes to enhancing biomass accumulation, increasing water uptake, protecting against drought, and mitigating stress due to noble metals.

- **RSLVs (Reactive short-chain leaf volatile):** They are oxylipins and are derived from PUFAs in the thylakoid membrane. Biologically, plants treated with vaporized RSLVs show an enhanced expression of genes involved in responding to environmental stresses, such as high temperatures and oxidative stress. RSLVs potentially act as signaling chemicals involved in heat and oxidative responses. A representative RSLV, 2-hexenal, is a green leaf volatile that induces gene expression in response to heat and oxidative stresses (Yamauchi et al., 2015) and thus enhances thermo tolerance in plants. The field use of 2-hexenal is being progressed commercially in Japan. A preliminary examination showed that its application in greenhouses improved the production of crops such as tomato, strawberry, and cucumber in the summer (unpublished data), suggesting that its use as a bio stimulant is effective in overcoming heat stress.

11.11 C_4 ENGINEERING

Abiotic stress is the major factor limiting photosynthetic activity, resulting in growth and yield reduction. The photosynthesis machinery also affects metabolic processes and oxidative stress regulation. Photosynthesis as the engine for life on earth has high engineering potential, which has not yet been fully exploited. Converting a C3 plant into a C4 is only one of the many possibilities of increasing photosynthetic rates in C3 plants (Ort et al., 2015). Most plants use the C3 pathway of photosynthesis that is compromised by gross inefficiencies in CO2 fixation. However, some plants use a super-charged photosynthetic mechanism called C4 photosynthesis. The C4 pathway is used by the most productive vegetation and crops on Earth. In addition to faster photosynthesis, C4 plants demand less water and less nitrogen. Overall, our aim is to introduce the characteristics of C4 into C3 crops. This would increase yield, reduce land area needed for cultivation, decrease irrigation, limit fertilizer applications and thus also provide abiotic stress tolerance in plants. If current C3 crops could be converted to use C4 photosynthesis, large economic and environmental benefits would ensue from both their increased productivity and the reduced inputs associated with the C4 pathway.

It is important to note that the huge advances in agricultural production associated with the Green Revolution were not associated with increases in photosynthesis, and so its manipulation remains an unexplored target for crop improvement both for food and biomass. C4 photosynthetic plants outperform C3 plants in hot and arid climates. By concentrating carbon dioxide around Rubisco C4 plants drastically reduce photorespiration. The frequency with which plants evolved C4 photosynthesis independently challenges researchers to unravel the genetic mechanisms underlying this convergent evolutionary switch. The conversion of C3 crops, such as rice, towards C4 photosynthesis which is a long-standing goal. Nevertheless, at the present

time, in the age of synthetic biology, this still remains a monumental task, partially because the C4 carbon-concentrating biochemical cycle spans two cell types and thus requires specialized anatomy. In recent years, efforts have been given to engineer C4 photosynthesis into C3 crops which we know as C4 engineering. The expression of genes encoding enzymes such as phosphoenolpyruvate carboxylase (PEPC), the chloroplastic pyruvate orthophosphate dikinase (PPDK), and NADP-malic enzyme (NADP-ME) into rice, tobacco and potato improved photosynthetic rate and yield.

Research efforts are also focused on obtaining Kranz anatomy, especially in rice which have an intermediate anatomical characteristics between C3 and C4 plants. Thus, in order to obtain C4 crops, new transformation methods together with additional efforts to better understand the function of C4 enzymes in a proper leaf anatomy are needed. Thus, in order to obtain C4 crops, new transformation methods are needed. Another important aspect that has to be addressed is source/sink relationships. From an evolutionary perspective C3 plants have modified their sink size proportionally to the source size (i.e. photosynthesis organs). Thus, more efficient carbon fixation via C4 pathway in the transformed plants would require to adapt the sinks to attain efficient harvest index. By step-wise identification of all of the components needed for engineering, it will eventually become possible to employ this powerful machinery to increase yields for the future.

11.12 SUMMARY

Abiotic stresses such as extreme temperatures (cold and heat), drought (water stress), excessive watering (Flooding/water logging), salinity and heavy metal toxicity negatively impact growth, development, yield and seed quality of crop and other plants. In future it is predicted that fresh water scarcity will increase and ultimately intensity of abiotic stresses will increase. Hence there is an urgency to develop crop varieties that are resilient to abiotic stresses to ensure food security and safety in coming years. A plants first line of defense against abiotic stress is in its roots. The chances of surviving stressful conditions will be high if the soil holding the plant is healthy and biologically diverse. One of the primary responses to abiotic stress such as high salinity is the disruption of the Na^+/K^+ ratio in the cytoplasm of the plant cell. The phytohormone abscisic acid (ABA) plays an important role during plant adaptation to environmental stress such as high salinity, drought and low temperature (Seki and Reddy, 2007). In order to increase abiotic stress tolerance in plants different chemical modulations and biochemical approaches have been imposed. C4 engineering is a technique which is another way out for providing abiotic stress tolerance in future.

11.13 GLOSSARY

Abiotic: Pertaining to non-living.

Anti-freeze proteins: The protein formed in the cells in response to freezing temperatures and bind to the surface of ice crystals and check their proliferation.

Chilling injury: Injuries to plants caused by low temperatures well above the freezing point of water.
Chlorosis: Yellowing of leaves of plants due to some mineral deficiency.
Compatible solutes: Organic compounds such as proline, glycinebetaine, sorbitol etc., which accumulate in cytosol in response to water stress or salt stress and contribute to osmotic adjustment of cells without damaging the cytosolic enzymes.
C_3 plants: Plants with C_3 cycle (calvin cycle) only in which first stable product of CO_2 fixation in photosynthesis is 3PGA (a 3-C compound) e.g. wheat, rice, barley etc.
C_4 plants: Plant with Kranz type of anatomy of leaves and Hatch-Slack pathway in which first stable product of photosynthetic CO_2 fixation is oxaloacetate (a 4-C compound) e.g. sugarcane, maize, sorghum etc.
Drought resistance: Capacity of a plant to limit and control consequences of water deficit.
Essential elements: Elements that are necessary for the normal growth and development of plants.
Freezing injury (Frost injury): Injury caused to plants by low temperature below the freezing point of water resulting in ice formation intercellularly or intracellularly.
Halophytes: Plants native to saline soil where they can grow well, compete with other soecies in the same habitat and complete their life cycle
Heat shock proteins (HSPs): A specific group of proteins produced in cells in response to rapid rise in temperature. They act as molecular chaperons thus, facilitating denatured or unfolded proteins to get back to their native conformation.
Ice nucleation: The beginning of ice crystal formation in response to freezing temperatures around some polysaccharides and proteins present in cell walls that act as ice nucleators.
Phytohormones: Organic substance produced naturally in the higher plants, controlling growth or other physiological functions at a site remote from its place of production and active in minute amounts.
Salinity: High concentration of total salts in the soil
Salinity stress (Salt stress): Adverse effects of excess mineral salts on plants.
Sink: A plant organ that does not produce enough photosynthates for its consumption and storage and imports them from other photosynthesizing parts (sources) of the plants. Examples; roots, tubers, young leaves and maturing fruit.
Stress: Adverse effect on growth and development of plant exerted by an external environment factor.

11.14 SELF ASSESSMENT QUESTION

11.14.1 Multiple choice questions:

1. Which is produced during water stress that brings stomatal closure
(a) Ethylene (b) Abscisic acid
(c) Ferulic acid (d) Coumarin

2. The study of functioning of plants under adverse environmental conditions is called as,
(a) Stress physiology (b) environmental physiology
(c) ecological physiology (d) None of the above

3. In cells of water stressed plant, the compatible solutes,
(a) lower the water potential of cells (b) lower the osmotic potential of cells
(c) do not interfere with enzymes functions (d) All of the above

4. Which of the following ions chiefly contribute to soil salinity
(a) Na^+, Cland HCO_3^- (b) K^+ and Ca^{2+}
(c) Mg^{2+} and SO_4^{2+} (d) All of the above

5. Saline soils are,
(a) beneficial to growth of crop plants (b) detrimental to growth of crop plants
(c) not of much concern in agriculture (d) None of the above

6. Which of the following amino acid chiefly accumulate in cells of water stressed and salt stressed plants to maintain osmotic relations of the cells
(a) Glycine (b) Proline
(c) Leucine (d) None of the above

7. External symptoms of chilling injury to plants are:
(a) Reduced growth (b) chlorosis and lesions on leaves
(c) appearance of foliage as if soaked in water for long (d) all of the above

8. Chilling injury in plants results due to,
(a) low temperature above freezing point (b) low temperature at freezing point
(c) low temperature below freezing point (d) none of the above

9. Which of the following hormone is involved in drought and frost resistance of plants
(a) ABA (b) IAA
(c) Ethylene (d) None of the above

10. Which of the following are more susceptible to chilling injury
(a) Tropical plants (b) Subtropical plants
(c) Both tropical and subtropical plants (d) Temperate zone plants

11. Heat-shock proteins were first discovered in,
(a) *Drosophila melanogaster* (b) *Arabidopsis*

(c) Cacti (d) none of the above

12. Lipid bilayers of cell membranes in chill-resistant species have,
(a) Higher proportion of saturated fatty acids (b) Higher proportion of unsaturated fatty acids
(c) Equal proportion of both (d) only saturated fatty acids

13. Which of the following is most susceptible to heat stress
(a) RUBISCO (b) Photosystem II
(c) Respiration (d) None of the above

14. Which of the following does not have cryoprotective function in plant tissues
(a) Antifreeze proteins (b) Sucrose
(c) Saturated fatty acids (d) none of the above

15. Accumulation of phytochelatins in plant cell vacuoles is indicative of,
(a) Salt stress (b) heavy-metal stress
(c) Heat stress (d) all of the above

16. Which of the following are considered as heavy-metals:
(a) Lead and Cadmium (b) Mercury and chromium
(c) Nickel, zinc and copper (d) all of the above

17. Relatively higher proportion of saturated fatty acids in membrane lipids is characteristic of
(a) High temperature sensitive plants (b) high temperature tolerant plants
(c) C_3 plants (d) All of the above

18. Heavy-metal toxicity in plants is causes,
(a) Increased lipid peroxidation in membrane (b) DNA damage
(c) oxidation of sulfhydryl groups of proteins (d) all of the above

19. Increased heavy-metal concentration in the environment is mainly,
(a) Natural (b) anthropogenic
(c) due to activities of microorganisms (d) none of the above

20. Which of the following is/are biochemical compounds that play role in plant resistance under stress
(a) Polyamines (b) RSLVs
(c) Nitrophenols (d) all of the above

11.14.2 Fill up the blanks:

1. Ambient temperature usually above_____ is considered as heat shock.
2. _____ are unique proteins produced in response to sudden increase in temperature.
3. _____ and _____ are two types of low temperature stress in plants.
4. Solidification of the cellular content into amorphous state is known as _____.
5. _____ and _____ are important morphological mechanisms followed by plants under drought stress.
6. _____ proteins are accumulated in plants under drought stress.
7. Anaerobic condition develops in root system of plants experiencing _____ stress.
8. In _____ submergence both shoot and root compartments of plants are under water.
9. Plants that can withstand salinity are known as _____.
10. _____ & _____ are the primary effects imposed on plants by salt stress.
11. _____ stabilizes photosystem II under high temperature stress.
12. Plants acclimation to freezing results in the synthesis of _____ proteins.
13. _____ is the common drought resistance mechanism in annual plants.
14. In flooding stress _____ formation is the main response to tolerance mechanism.
15. _____ under cold stress serves as cryoprotectants to save the plants.

11.14.3 True or False:

1. Heat shock proteins are produced in response to high temperature.
2. Compatible solutes help in osmotic adjustment of cell under stress.
3. Chilling stress is more lethal than freezing stress.
4. Heat shock proteins were first discovered in fruit fly.
5. Drought avoidance allows plants to end its life cycle before the onset of harsh conditions.
6. Dehydrins and LEA proteins accumulate under drought conditions in plants.
7. Glycophytes are the plants that cannot withstand salinity and die.
8. Metallotioneins are heavy-metal chelating ions produced under heat stress.
9. Osmolytes are toxic substances that accumulate in plants under stress conditions.
10. C_4 engineering deals with the photosynthetic pathway of C_3 and C_4 plants.
11. Phytohormones play important role in abiotic stress tolerance
12. Stress is defined as any internal unfavourable condition that adversely affects plant's life.
13. Abiotic stress include non-living factors that affecting plant growth and development.
14. Exogenous application of thiourea stimulates defense mechanism in plants under stress.
15. RSLV is a volatile compound that enhances thermotolerance in plants.

11.14.4 Very short answer questions:

1. Name the proteins that accumulate under heat stress.

2. What are the different effects shown by plants under heat stress?
3. Name two different types of low temperature stress and define them.
4. Name four chemical and biochemical compounds that results abiotic stress tolerance.
5. Define osmolytes and name different osmolytes that accumulates under stress condition.
6. Define glycophytes and halophytes.
7. Define cryoprotectants and antifreeze proteins
8. Define compatible solutes and their role in plants
9. Explain partial and complete submergence.
10. Define the following abbreviations:
11. (a) RSLVs (b) LEA (c) DREB

11.14.1 Answer key: 1-b, 2-a, 3-d, 4-d, 5-b, 6-b, 7-d, 8-a, 9-a, 10-c, 11-a, 12-b, 13-b, 14-c, 15-b, 16-d, 17-b, 18-d, 19-b, 20-d.

11.14.2 Answer key: 1-10-15°C, 2-Heat shock proteins, 3-Chilling, freezing, 4-Vitrification, 5-Drought escape, Drought avoidance, 6-LEA, 7-Flooding, 8-Complete, 9-Halophytes, 10-Osmotic stress, ion toxicity, 11-Glycinebetaine, 12-Antifreeze, 13-Drought avoidance, 14-Aerenchyma, 15-Osmolytes.

11.14.3 Answer key: 1-True, 2-True, 3-False, 4-True, 5-False, 6-True, 7-True, 8-True, 9-False, 10-True, 11-True, 12-False, 13-True, 14-True, 15-True.

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11.16 SUGGESTED READINGS

- Plant Physiology by Teiz & Zeiger.
- Fundamentals of plant physiology by V.K. Jain.

- Plant Physiology by Salisbury and Ross.
- Plant stress physiology by P.C. Trivedi.
- Plant Physiology by Pandey and Sinha.

11.17 TERMINAL QUESTIONS

11.17.1 Short answer questions:

1. Define drought and flooding stress.
2. Define metal stress and the role of phytochellatins under metal stress.
3. Name different types of abiotic stress and define them.
4. Write short note on;
   (a) Phytochellatins (b) HSPs (c) Osmolytes (d) Cryoprotectants
5. What are the chemical approaches for abiotic stress tolerance in plants?
6. Write a note on chilling stress.
7. Differentiate between acclimation and adaptation
8. Write a note on C_4 engineering.
9. Explain the mechanism of heavy metal tolerance in plants
10. Write a note on RSLVs and explain its role as a biostimulant.

11.17.2 Long answer question:

1. Describe water stress and their physiological effects on plants.
2. Define temperature stress and explain different mechanism of its tolerance in plants.
3. Explain the role of different chemical and biochemical compounds in stress tolerance.
4. Define stress and explain different mechanisms of abiotic stress tolerance in plants.
5. Discuss the role of:
   a. HSPs in thermo tolerance
   b. Phytohormones in drought
   c. ROS in abiotic stress
   d. Carbohydrates and lipids in stress tolerance

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